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Biologically Active Zone Enhancement (BAZE) Supplemental Study: Mass Balance of RDX Biotransformation and Influence of Aquifer Temperature on RDX Biodegradation in Groundwater

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ABSTRACT: A series of column studies with site-specific aquifer material from the former Nebraska Ordnance Plant were performed to evaluate the influence of aquifer temperature on in situ hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) biodegradation, and to assess the ultimate fate of RDX in groundwater under biologically induced reductive conditions. In treatment columns RDX-contaminated water was amended with acetate as readily available carbon source, and in control columns no electron donor was used. The results of the temperature study demonstrated clear indications of adverse effects of lower aquifer temperature on biological activity of RDX-degraders. As the aquifer temperature decreased from 15 to 10 and eventually to 5 °C, the concentration of nitroso-substituted metabolites and untreated RDX increased in the effluent stream. The estimated first-order biodegradation rate coefficient *k* for RDX at 15 °C was 0.155 1/hr (±0.019, n = 3). This rate coefficient decreased by about 37 percent to 0.098 1/hr (±0.017, n = 3) at 10 °C, and by another 38 percent to 0.061 1/hr (±0.016, n = 3) at 5 °C. An activation energy of 63.54 kJ/mol RDX was estimated from these reaction rate coefficients at three different aquifer temperatures. Results of the radiolabel study demonstrated that the ultimate fate of RDX under in situ reductive conditions is highly dependent on redox conditions in the aquifer. In treatment columns (redox change, ΔE_h = -550 to -700 mV), 23-46 percent of initial radiocarbon was mineralized to $^{14}CO_2$ compared with <5 percent in control columns, where ΔE_h ranged between 70 and -70 mV. The dissolved fraction of initial radiocarbon in treatment columns was estimated between 46 and 64 percent. No or very low levels of nitroso-substituted RDX transformation products were identified in dissolved fraction from treatment columns. In control columns dissolved fraction accounted for about 86 percent of initial ^{14}C and was composed of mainly untreated RDX.

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Preface

This research work is a supplemental study for the biologically active zone enhancement (BAZE) treatability study for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) biodegradation in groundwater. The purpose of this research project was to determine the influence of aquifer temperature on in situ biodegradation of RDX in a biologically induced reductive environment. The study also evaluated the mass balance of RDX biotransformation in groundwater using radiolabeled RDX. A series of column studies was conducted using aquifer material from the former Nebraska Ordnance Plant (NOP), Mead, NE.

The research was conducted at the Environmental Laboratory (EL), Vicksburg, MS, U.S. Army Engineer Research and Development Center (ERDC). The funding for this project was provided by the Environmental Security Technology Certification Program (ESTCP), under BAZE project ESTCP CU-0110.

This report was prepared by Dr. Altaf H. Wani and Ms. Deborah R. Felt, Applied Research Associates, Inc., Vicksburg, MS; and Dr. Jeffrey L. Davis, Environmental Engineering Branch (EEB), Environmental Processes and Engineering Division (EPED), EL. Ms. Brenda O'Neal, EEB, provided analytical assistance throughout the research project.

This study was conducted under the direct supervision of Dr. Patrick N. Deliman, Chief, EEB, and Dr. Richard E. Price, Chief, EPED, and under the general supervision of Dr. Elizabeth C. Fleming, Acting Director, EL.

Commander and Executive Director of ERDC was COL James R. Rowan, EN. Director was Dr. James R. Houston.

1 Introduction

A large number of active and formerly used military installations are contaminated with explosive polynitroorganics. The most common munition-derived pollutants encountered at these sites are nitroaromatics like 2,4,6-trinitrotoluene (TNT), and nitramines such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-tetrazocine (HMX). These explosive compounds have entered the environment from sites where they were manufactured, stored, disposed, or used in military training. Currently, there are 583 sites with confirmed explosives-contaminated groundwater at 82 installations nationwide; and at 22 other installations, 88 additional sites are suspected of groundwater contamination with explosives and organics (Defense Environmental Network and Information Exchange (DENIX) 2002).

RDX, a cyclic nitramine explosive, has contaminated groundwater, soil, and surface water at many military installations, promoting concerns about potential toxic effects. A previous treatability study (Wani et al. 2002) has shown that in situ bioremediation of RDX can be achieved by inducing a reductive environment using a benign carbon source (electron donor) in the aquifer. Among different electron sources tested, acetate as a carbon amendment resulted in the necessary reduced conditions for RDX biotransformation without the generation of toxic byproducts. The prior treatability study was conducted at room temperature (22 ± 1 °C), and the influence of lower aquifer temperatures (8-10 °C) on RDX biotransformation kinetics was not evaluated. In addition, the treatability study indicated no formation of nitroso-substituted products and complete RDX (~ 100 μg/L) removal from the groundwater. It was hypothesized that the ultimate fate of RDX under such in situ conditions appears to be nonvolatile non-nitroso transformation products. To back up this hypothesis and to evaluate the ultimate fate of RDX under reductive biotransformation, a radiolabel RDX study was performed. The prior study resulted in two unresolved issues: (a) the influence of aquifer temperature on RDX biotransformation kinetics and (b) the ultimate fate of RDX under in situ bioremediation. Because of these two unresolved issues, a supplemental study was conducted to (a) evaluate the influence of aquifer temperature on in situ RDX biodegradation and (b) assess the ultimate fate of RDX in groundwater under biologically induced reductive conditions.

2 Literature Review

TNT, RDX and HMX, the most commonly encountered energetic contaminants in soil and groundwater, pose a significant cleanup challenge at many active and formerly used military sites in the United States and across the world. In the United States the contamination of soil and groundwater is attributed to World War II and the Korean conflict (Pennington 1999).

RDX, which is in the nitramine class of explosives, is widely used in munitions because of its explosive power, around 1.5 to 2 times that of TNT, and rapid detonating velocity, about 1.3 times that of TNT (U.S. Army 1984). RDX is of particular environmental concern because laboratory studies have established that it is generally resistant to microbial transformation in aerobic soils (McCormick et al. 1981) and it is not extensively sorbed on soils (sorption coefficient K_d of 0.83 to 0.95 L kg^{-1}) (Singh et al. 1998, Sheremata et al. 2001). Remediating soil and water contaminated with RDX is of vital importance because ingestion of RDX can adversely affect the central nervous system, gastrointestinal tract, and kidneys. Common symptoms of RDX intoxication include nausea, vomiting, hyperirritability, headaches, and unconsciousness (Eitner 1989). RDX has also been associated with systemic poisoning usually affecting bone marrow and the liver (Agency for Toxic Substances and Disease Registry (ATSDR) 1996). The U.S. Environmental Protection Agency (EPA) has established a drinking water health advisory of 2 μg/L for exposure to RDX (U.S. EPA 2002).

The fate and transport of RDX in the environment are influenced by many factors including photolysis by sunlight, hydrolysis, and biologically mediated degradation. Biodegradation of RDX is often attributed to cometabolism in the presence of a primary carbon source under various electron acceptor conditions. RDX can be biodegraded under anaerobic or anoxic conditions by facultative or anaerobic microorganisms (McCormick et al. 1981; Kitts et al. 1994; Freedman and Sutherland, 1998; Hawari et al. 2000a; Halasz et al. 2002; Beller 2002). Under aerobic conditions, RDX can be used as a sole source of nitrogen by aerobic microorganisms (Binks et al. 1995; Coleman et al. 1998; Brenner et al. 2000), or by fungus (Bayman et al. 1995; Fernando and Aust 1991; Sheremata and Hawari 2000).

Various laboratory studies have established that anaerobic RDX metabolism occurs more readily than aerobic metabolism, and that hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) are the transient

biotransformation intermediates (Figure 1) under anaerobic conditions (Hawari et al. 2000a, 2000b; McCormick et al. 1981; Kitts et al. 1994; Morley et al. 2002; Young et al. 1997; Beller 2002; Freedman and Sutherland 1998; Beller and Tiemeier 2002). Recent studies have tentatively identified methylenedinitramine (MDNA) as the ring cleavage metabolites during the bioremediation of RDX with anaerobic sludge. These studies suggest different views of the stability of MDNA; it can occur as a transient metabolite (Halasz et al. 2002) or as a persistent transformation product that appears at substantial concentrations relative to RDX (Oh et al. 2001). Nonetheless, Beller and Tiemeier (2002) reported that under in situ conditions MDNA was not detected in any of the samples from the RDX-contaminated aquifer at Iowa Army Ammunition Plant (IAAP), although relatively high concentrations of MNX, DNX, and TNX were present.

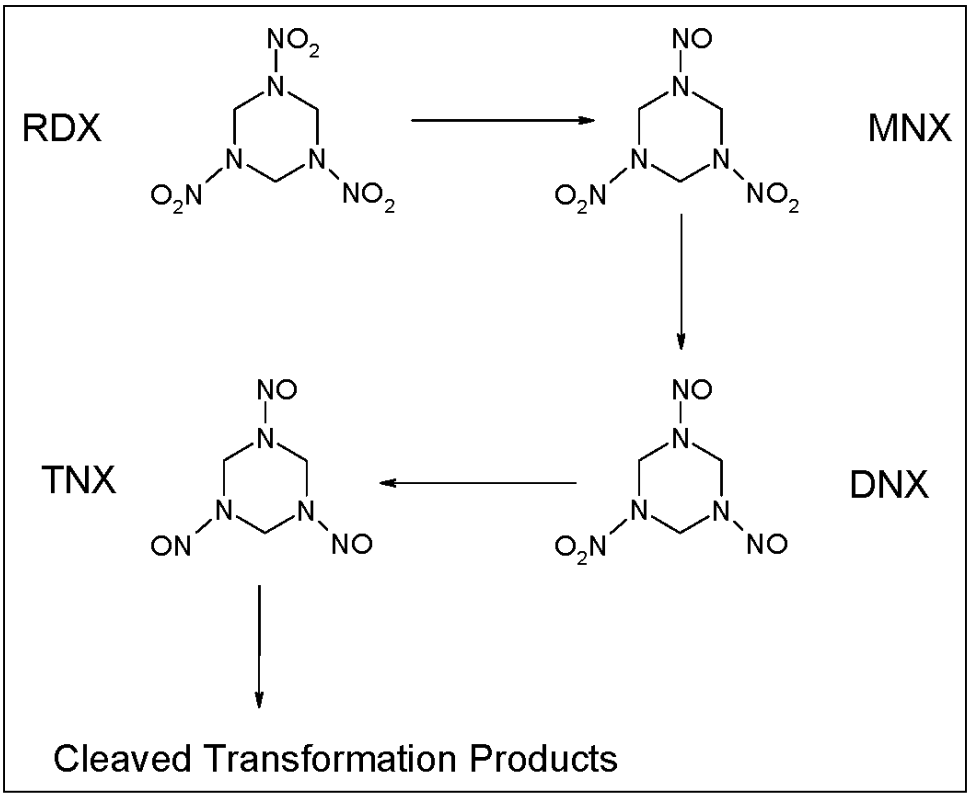

Figure 1. Anaerobic pathway

Although many researchers have established that RDX can be biodegraded through biological processes, successful application of these techniques to in situ treatment of contaminated soils and waters has yet to be proven in the field. The influence of such environmental conditions as aquifer temperature on RDX biodegradation has not been considered in previous research work. Moreover a better understanding of in situ biotransformation of RDX and the generation of transformation products requires the assessment of the ultimate fate of RDX.

3 Site Description and Sampling

The former Nebraska Ordnance Plant (NOP) is located about 1 km (half a mile) south of Mead, NE, which is 48 km (30 miles) west of Omaha and 56 km (35 miles) northeast of Lincoln, NE. The NOP covers 69.9 square km (17,258 acres) in Saunders County. Currently, the land is owned by the University of Nebraska, Agricultural Research and Development Center, U.S. Army National Guard and Reserves, U.S. Department of Commerce, and private interests. The operational history and geological and hydrological characteristics of the NOP site are discussed in Wani et al. (2002).

Aquifer material at the former NOP site was collected from Area 1, near monitoring well MW-5B, from a depth of 11 to 12 m (36 to 40 ft) below ground surface. Soil columns were collected in 5-cm- (2-in.-) diameter acetate liners by the direct-push method using a track-mounted mobile sampling device. Further details on aquifer material sampling are presented in the biologically active zone enhancement (BAZE) treatability study report (Wani et al. 2002). The soil columns were thoroughly sealed at both ends to prevent loss of water from the aquifer material during storage and shipping. Samples of aquifer material were transported to the Environmental Laboratory, Vicksburg, MS, U.S. Army Engineer Research and Development Center, via a refrigerated truck.

4 Materials and Methods

Experimental Setup

Two sets of triplicate columns were used to evaluate the effects of aquifer temperature on RDX biotransformation. In the first triplicate set, acetate was added as the carbon source (electron donor) while the second triplicate set served as amendment (carbon source) control. The polyvinyl chloride (PVC) columns were 104 cm (3.4 ft) long with an inside diameter of 3.8 cm (1.5 in.). Both ends of the columns were closed with PVC caps screened with porous (100 μm) PVC. Additional sampling ports, at 26 cm (10.2 in.), were placed along the entire column length resulting in three intermediate sampling ports in addition to the inlet and outlet ports for the development of the contaminant bed profile. Each column was individually wrapped with a thermal jacket composed of a cold-water circulation unit covered with a 12-mm (0.5-in.) thick thermal insulation to prevent heat transfer from the environment. The difference in influent and effluent temperature was ± 1 °C. The detailed design of the column system with groundwater flow and other instrumentation is shown in Figure 2. Teflon-coated T-type thermocouples (Omega Engineering, Stamford, CT) equipped with digital panel monitors were installed at the inlet and outlet of each column, via flow-through cell, to record the temperature of influent and effluent groundwater streams. Pressure gauges were installed at the inlet to each individual column to examine the effects of microbial growth (biofouling) on groundwater flow, back pressure, and the hydrodynamic properties of the aquifer material. The outlet of each column was equipped with an oxidation-reduction potential (ORP) electrode via a flow-through cell to compare the reduced conditions along the column length with that of the inlet tank. Details on packing of these columns with site-specific aquifer material were presented in the initial BAZE treatability study (Wani et al. 2002). RDX-contaminated water was pumped through the columns using variable-control positive displacement pumps. Variable control on pump speed allowed the metering of desired water flow through each column.

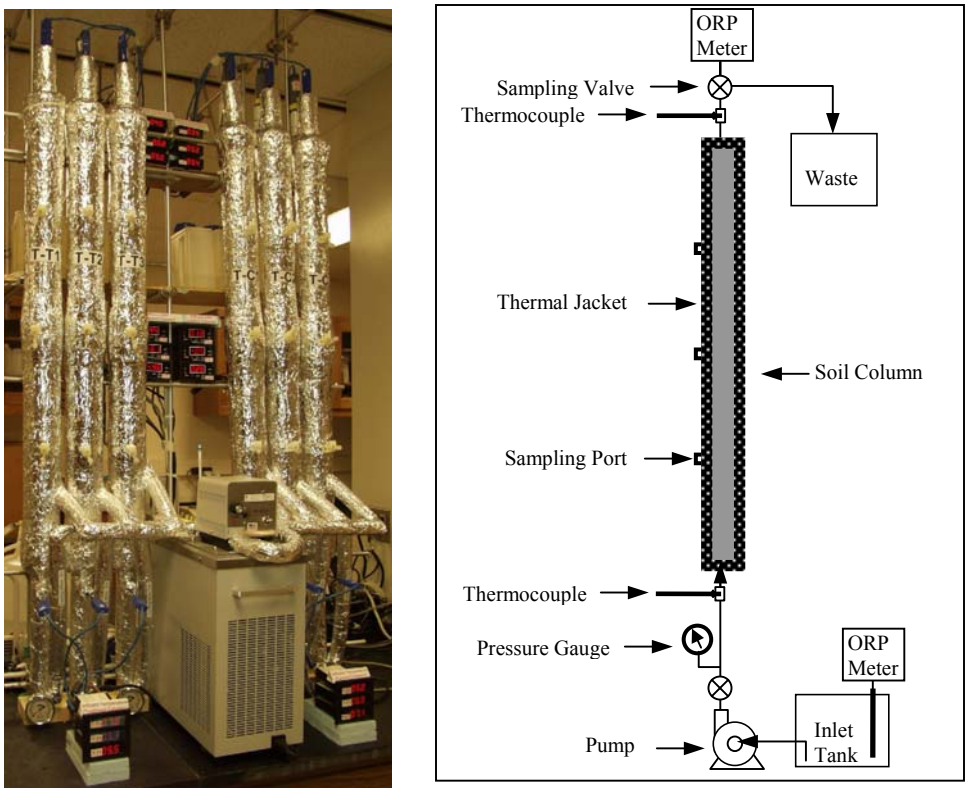

Figure 2. Experimental column setup for temperature study

To assess the ultimate fate of RDX in groundwater under a biologically induced reductive environment, two separate sets of triplicate columns, as shown in Figure 3, were used. Similar to the temperature study, one set was used for amendment (carbon source) addition and the other set served as amendment (acetate) control. These PVC columns were of the same dimensions as described for the temperature study. These columns also had additional sampling ports at 26 cm (10.2 in.) for bed profile analysis. The schematics of this column system with RDX-contaminated water flow and other instrumentation are illustrated in Figure 3. Pressure gauges were installed at the inlet to each individual column to examine the changes in back pressure. The outlet of each column was equipped with an ORP electrode via a flow-through cell to compare the reduced conditions along the length of the column system with that of the inlet tank. These columns were packed with site-specific aquifer material (Wani et al. 2002). RDX-contaminated water was pumped through each column using variable-control positive displacement pumps. The column system along with pumps and inlet water reservoirs was securely installed in a cabinet to prevent release of any radioactivity.

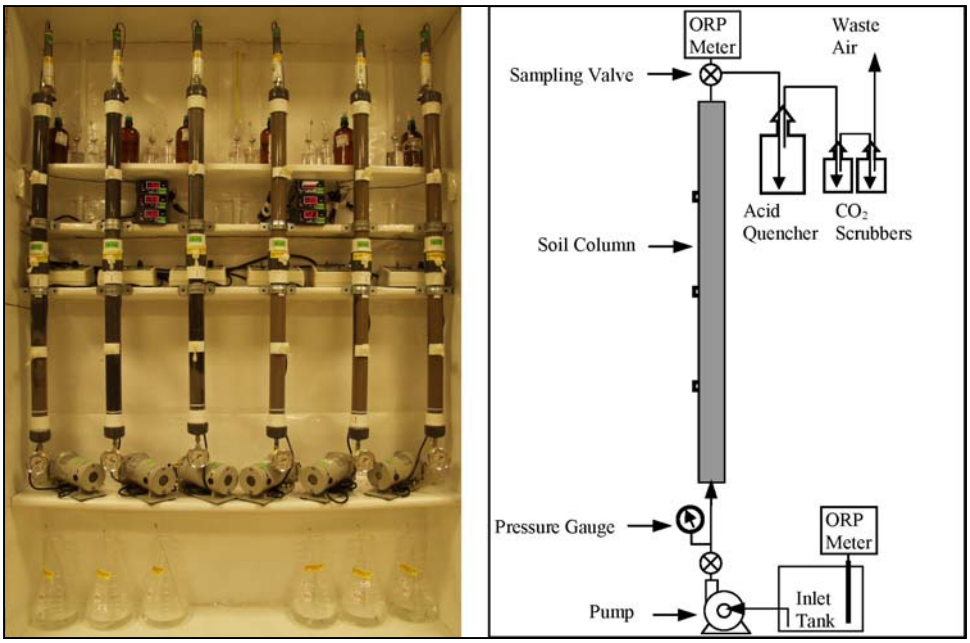

Figure 3. Experimental column setup for radiolabel RDX study

Operation

RDX-contaminated water was prepared by spiking autoclaved organic-free reagent-grade water with RDX stock solution. RDX-contaminated water with a concentration of about 1.03 ± 0.05 mg/L was used in this study. The selection of acetate as the carbon source (electron donor) in this research work is based on other research that suggests that acetate is an excellent electron donor to stimulate in situ microbial reductive conditions (He et al. 2002; Wani et al. 2002). Acetate concentration of 500 mg/L (as carbon) was used in both temperature- and radiolabeled-studies to ensure that organic carbon is not the limiting factor. RDX-contaminated water flow through each column was initiated at ~ 0.2 mL/min and maintained at this rate throughout the study. This water flow resulted in a velocity of about 0.85 m/day (2.7 ft/day), which is comparable with the NOP site groundwater velocity of approximately 0.61 m/day (2 ft/day).

Temperature-study columns were operated at three different temperatures (15, 10, and 5 °C) to evaluate the influence of aquifer temperature on RDX biotransformation kinetics. Each temperature test lasted for a month. Liquid samples were collected from inlet and outlet sampling ports every fifth day. After the columns reached the steady state, samples from intermediate ports along the column height were collected on the 23rd and 30th day for each temperature test. Water samples were stored at 4 °C until explosives and amendment analysis. The operating conditions are summarized in Table 1.

Table 1
Column Operating Conditions

Column	Groundwater Flow Rate, mL/min	RDX Concentration mg/L	Acetate Concentration mg/L C	[^{14}C]RDX Initial Activity, dpm
Temperature Columns				
T-T1	0.20	~1.0	~500	None
T-T2	0.20	~1.0	~500	None
T-T3	0.20	~1.0	~500	None
T-C1	0.20	~1.0	0	None
T-C2	0.20	~1.0	0	None
T-C3	0.20	~1.0	0	None
Radiolabel RDX Columns				
R-T1	0.20	~1.0	~500	~1,700,000
R-T2	0.20	~1.0	~500	~1,700,000
R-T3	0.20	~1.0	~500	~1,700,000
R-C1	0.20	~1.0	0	~1,700,000
R-C2	0.20	~1.0	0	~1,700,000
R-C3	0.20	~1.0	0	~1,700,000

Note: Amendment concentrations are nominal.
dpm = disintegrations per minute (µCi = 2.2 million dpm)

Radiolabeled-study columns were fed with RDX-contaminated (1.05 ± 0.06 mg/L) groundwater for 2 months to reach steady state. Once the columns reached steady state conditions with steady RDX removal from feed water, a slug of [^{14}C]RDX (~ 0.76 µCi) was introduced into the inlet tank to each individual column. The effluent water stream, including any carbon dioxide evolved as a result of mineralization, was collected in a 500-mL glass sampler under a hydrochloric acid quenching solution (25 mL, 1N HCl) to release any dissolved carbon dioxide. The effluent gases from the acid quencher were passed through carbon dioxide scrubbers containing 100 mL Carbo-Sorb® (Packard Biosciences, Meriden, CT) to scrub out carbon dioxide from the gas stream. In another test it was found that Carbo-Sorb is a very efficient carbon dioxide scrubbing solution with a 99.99 percent recovery. At the end of the sampling, the sampling train (acid quencher-Carbo-Sorb scrubbers) was flushed with nitrogen gas to remove all the carbon dioxide from the acid quencher into the Carbo-Sorb scrubbers (Figure 4).

The contents of the acid quencher (including the column effluent) were filtered (0.45 µm) to separate the suspended, mostly biomass (residue) and the dissolved (filtrate) fractions of RDX and its transformation products. The filtrate was neutralized with 1N NaOH. Liquid scintillation counting (LSC) was performed on aliquots from both the neutralized filtrate (dissolved fraction) and the residue (suspended fraction) to estimate the portion of [^{14}C]RDX and its transformation products in the suspended and dissolved phases. A 4-mL aliquot of neutralized filtrate was mixed with 15 mL Ultima Gold® (Packard Biosciences) scintillation cocktail for radioactivity counting. The filter paper along with the residue was immersed in 15 mL Ultima Gold® scintillation cocktail for radioactivity counting. The contents of the Carbo-Sorb scrubbers

were subjected to LSC to evaluate the fraction of [^{14}C]RDX mineralized to [^{14}C]CO_2. A 10-mL aliquot from Carbo-Sorb scrubber was mixed with 10 mL Permafluor® scintillation cocktail (Packard Biosciences) for radioactivity counting. The total radioactivity from gaseous (mineralization CO_2), suspended (nitroso- and non-nitroso-substituted nonvolatile metabolites), and dissolved (nitroso- and non-nitroso-substituted nonvolatile metabolites) phases was summed up and compared with the initial radioactivity introduced as [^{14}C]RDX. An aliquot from neutralized filtrate was analyzed for untreated RDX and nitroso-substituted (MNX, DNX, and TNX) nonvolatile metabolites using high-performance liquid chromatography (HPLC).

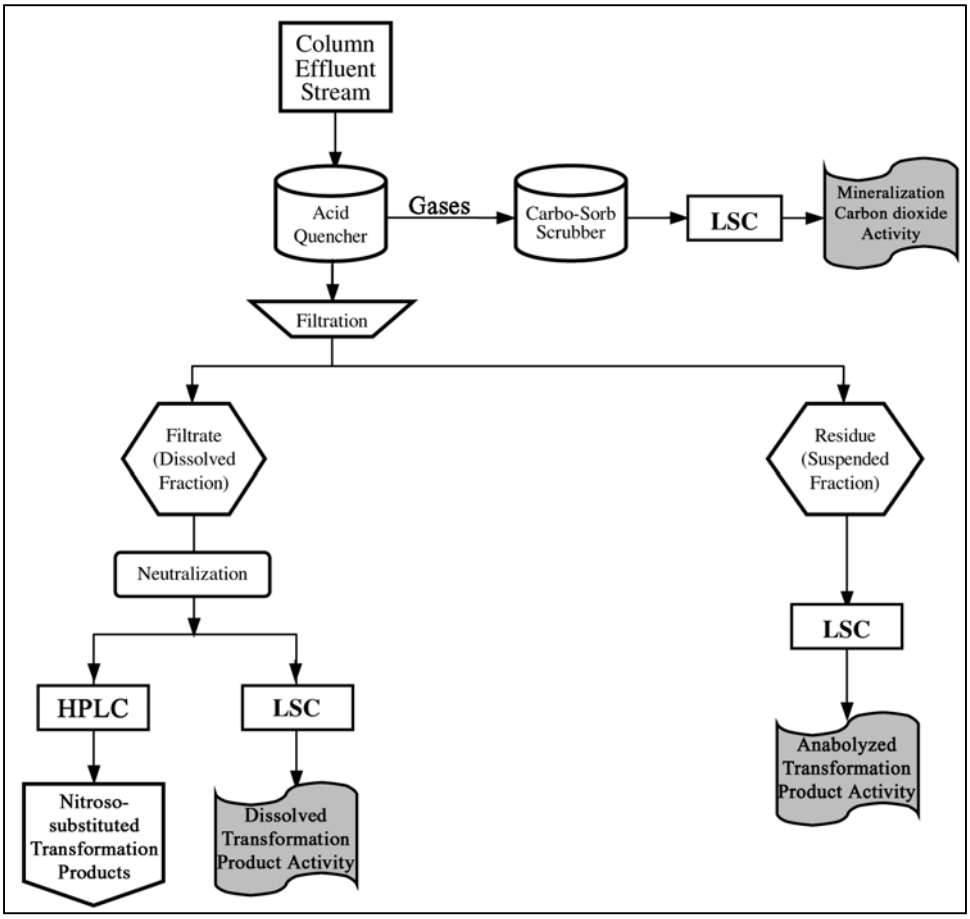

Figure 4. Radiolabel RDX sample preparation and analysis flow chart

Analytical Techniques

Acetate, sulfate, nitrate, and nitrite in liquid samples were analyzed on a Dionex (Sunnyvale, CA) Ion Chromatograph. Chemical separation and detection were achieved using an Ionpac AS11 analytical column (4 by 250 mm) and a Dionex conductivity detector (1.25 µL internal volume). The mobile phase consisted of NaOH at a flow rate of 1.5 mL/min. The sample volume was 25 µL of filtered (0.45 µm) sample. The instrument was calibrated daily from standards prepared from stock solutions. Check standards were run after every 10 samples.

The analysis of RDX and its nitroso-substituted transformation products was performed using a Dionex HPLC system comprising a P580 fluid pump, ASI-100 autosampler, and UVD340U absorbance detector. The injection volume was 25 µL. Chemical separation was achieved using a Supelco (Bellefonte, PA) CN reverse-phase HPLC column (25 cm by 4.6 mm). The mobile phase comprised 1:3 (volume per volume) methanol/organic-free reagent water at a flow rate of 1 mL/min. Explosives absorbance was monitored at 245 nm. For EPA Method 8330 analytes (U.S. EPA 1994), a seven-point calibration curve was used. The instrument was calibrated daily from standards prepared from stock solutions. Check standards were run after every 10 samples.

Sample radioactive concentration via liquid scintillation counting was done on a 2500 TR Packard Scintillation Counter (Packard Biosciences). The counter was equipped with a barium external source to enable correction for machine efficiency. The liquid scintillation protocol collected data up to 156 meqV, which is the maximum energy for [^{14}C]. Each sample was counted twice for 2 minutes.

Oxidation-reduction potential E_h and pH were measured with electrodes that were calibrated weekly. Both ORP and pH were measured with Oakton WD-35100-00 model pH/ORP Controllers (Cole-Parmer, Vernon Hills, IL) with a measuring range of 0 to 14 for pH and –1250 to 1250 mV ORP. ORP was measured using a Cole-Parmer combination redox electrode with platinum sensing surface and Ag/AgCl reference electrode. The value E_h was obtained by adding standard potential of the reference electrode E_R to the measured potential E. For this ORP electrode E_R at 25 °C (room temperature) is 202 mV. pH was determined with a Cole-Parmer combination electrode.

Biotransformation Kinetics

The rate of RDX biotransformation was determined by sampling at the intermediate ports in the column system. A contaminant profile was developed and an advection-dispersion model (Equation 1) for contaminant transport with decay was fitted to the results:

$$\frac{\partial C}{\partial t} = \alpha v \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - kC \qquad (1)$$

where

C = RDX concentration, mg/L

t = time elapsed, hr

α = dispersivity, cm

v = interstitial velocity, cm/hr

x = distance from column inlet, cm

k = RDX first-order biodegradation rate coefficient, 1/hr

With the boundary conditions $C(0,t) = C_0$ and $\partial C/\partial x(\infty,t) = 0$, at steady state, Equation 1 can be solved to Equation 2 as follows:

$$C = C_0 \cdot \exp\left[\left(\frac{x}{2\alpha v}\right)\left(v - \sqrt{v^2 + 4k\alpha v}\right)\right] \quad (2)$$

The bed-profile sampling for each temperature test was done twice on the 23rd and 30th days when the operating conditions were steady and columns had reached equilibrium conditions with steady RDX removal.

The rates of RDX biotransformation, estimated by fitting Equation 2 to the contaminant profile, at three different temperatures (15, 10, and 5 °C) were used to evaluate the influence of aquifer temperature on RDX biodegradation rate using the Arrhenius equation:

$$k = A \cdot \exp\left[\frac{-E_a}{RT}\right] \quad (3)$$

where

A = Arrhenius constant

E_a = activation energy, J/mol

R = universal gas constant, J/mol-K

T = temperature, °K

5 Results

Temperature Study

Column hydrodynamics

RDX-contaminated water flow during the entire 13-week study was approximately 0.2 mL/min in both triplicate column sets (Figure 5). This water flow resulted in an hydraulic residence time of 24 ± 1 hr in individual columns.

Figure 5 summarizes the groundwater temperature in each column. These temperature readings are the average of influent and effluent groundwater temperatures. The thermal jacket wrapped over the individual column was very efficient in maintaining the aquifer material and groundwater temperature in each column. The influent and effluent temperatures varied by 1 °C.

Reduced conditions were established in each column as shown in Figure 5. In treatment columns change in redox ΔE_h was between –600 and –850 mV. Anaerobic conditions were established in treatment columns by providing carbon source to indigenous microorganisms, which then used the oxygen, creating a reduced environment. The ΔE_h in the control columns was very small (between 100 and –150 mV) compared with that of the treatment columns.

The influent stream pH varied between 6.5 and 7.5 for the treatment column set where RDX-contaminated water was amended with acetate (Figure 6). In the control column set the influent water pH was slightly higher (7.5 to 8). The effluent stream from the treatment column set showed a slight increase in pH (7.5 to 8). There was no measurable change in the effluent stream pH in the control columns (Figure 6).

There was no significant back pressure buildup due to biofouling in any of the columns, and head loss remained almost the same during the entire 13-week study, except in treatment column T-T2 (Figure 6). This steady increase in back pressure in Column T-T2 could be the result of a higher biomass yield that caused RDX biodegradation without the detection of any nitroso-metabolites (Figure 7). Occasional hikes in the back pressure were due mainly to plugging of the porous PVC screen at the column inlets due to extracellular secretions from biomass. After the porous PVC screens were cleaned or replaced, this flow resistance was removed and pressure loss across the columns dropped to initial levels.

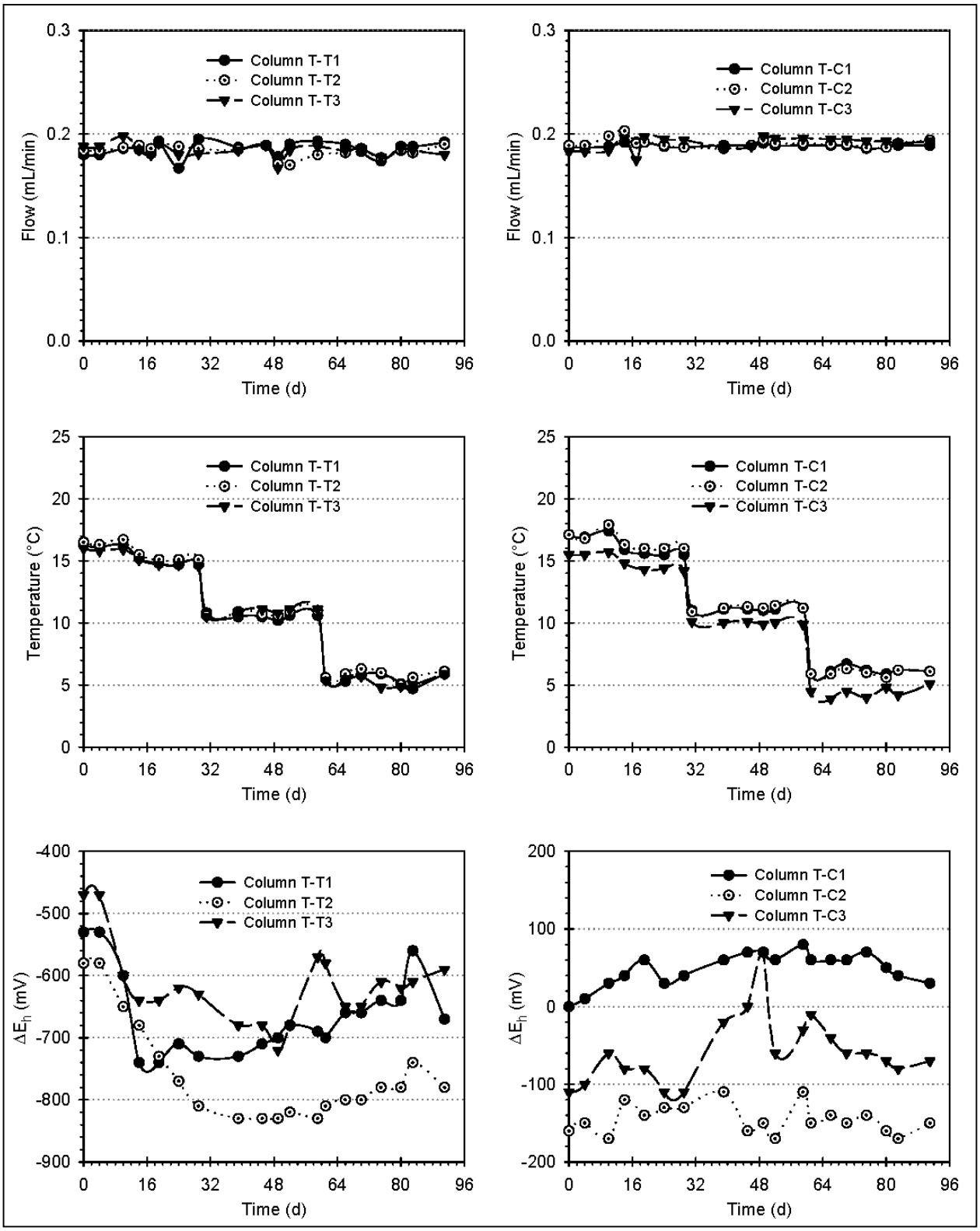

Figure 5. RDX-contaminated water flow, temperature, and change in redox potential for each column

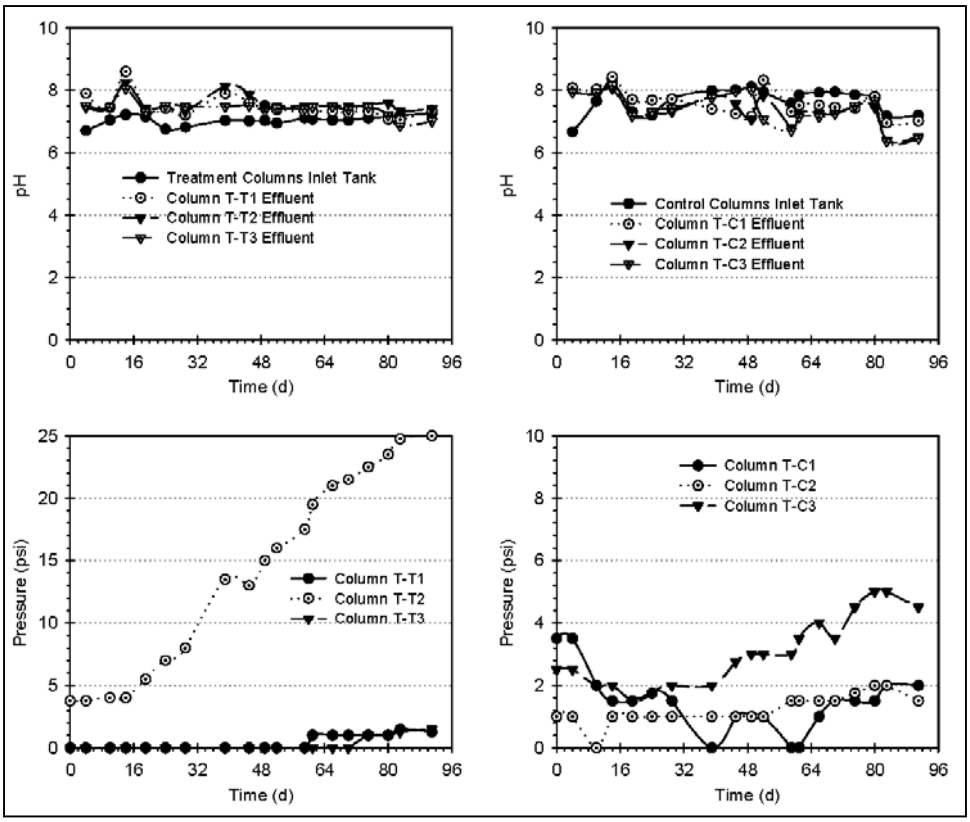

Figure 6. Feed water pH and flow resistance (back pressure) for each column (note: to convert pressure to kilopascals, multiply by 6.9)

RDX biotransformation

RDX concentrations in the influent groundwater, ranging between 1 and 1.2 mg/L, were reduced to below detection limits of 0.02 mg/L at 15 °C in all treatment columns. At lower temperatures (10 and 5 °C) low concentrations of RDX were observed in the effluent streams from Columns T-T1 and T-T3. However, these lower temperatures did not have any effect on the removal efficiency of RDX in Column T-T2. In Column T-T2 influent RDX was removed without the presence of any nitroso-substituted RDX metabolites at all three temperatures tested. In the other two treatment columns (T-T1 and T-T3) low levels (~ 0.2 mg/L) of the nitroso-substituted transformation products (MNX, DNX, and TNX) were observed in the effluent stream throughout the study (Figure 7). The other noticeable difference in Column T-T2 compared with Columns T-T1 and T-T3 was the steady back pressure development during the 13-week study. One plausible reason behind these two manifest observations in Column T-T2 could be a higher biomass yield that caused RDX biodegradation without the detection of any transformation products and at the same time created a higher flow resistance resulting in higher back pressure along the column length. The assumption of high biomass yield is also substantiated by the lowest redox potential in Column T-T2 as a result of higher biological activity. The cumulative presence of nitroso-substituted transformation products in Column T-T1 and Column T-T3 accounted for about one-third of the influent RDX concentration on a molar basis. That leaves about 70 percent of the inlet

RDX unaccounted for in terms of nitroso-substituted RDX intermediates, which might include other non-nitroso-transformation products as proposed by other researchers (Hawari et al. 2000a; McCormick et al. 1981).

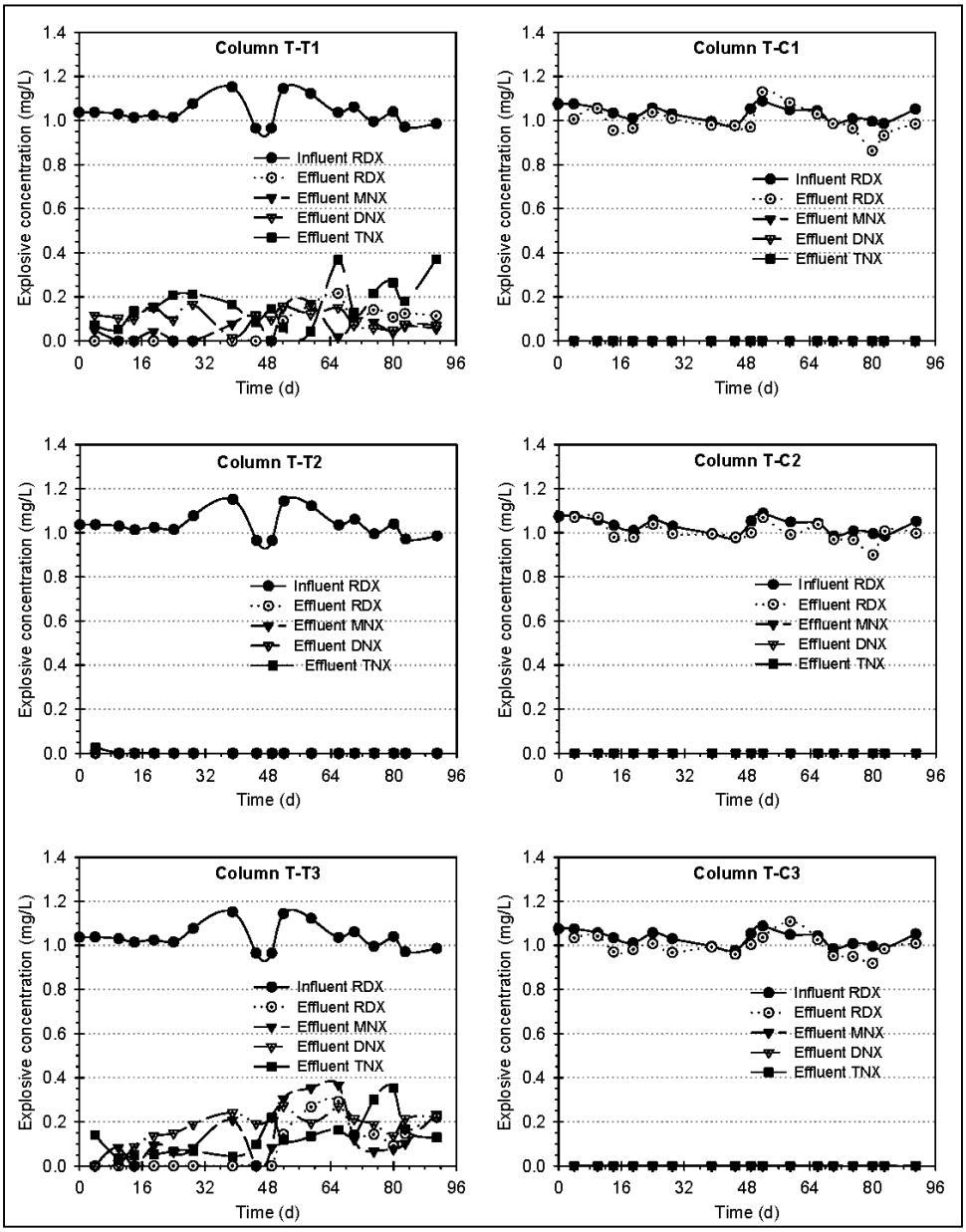

Figure 7. RDX and nitroso-RDX intermediates concentration in influent and effluent streams

In control columns no biodegradation of RDX was observed throughout the course of the study (Figure 7). During the entire study redox potential in control columns, where no electron donor was used, was very high compared with that of treatment columns (Figure 5). These results identify the need for low redox environment for reductive biotransformation of RDX in groundwater.

During the 13-week study, RDX was removed from the groundwater with the presence of low levels of all three nitroso-substituted transformation products in treatment Columns T-T1 and T-T3; however, in treatment Column T-T2 effluent no MNX, DNX, or TNX was observed. This sequential reductive biotransformation has been reported for various RDX-metabolizing cultures that used organic electron donors (Freedman and Sutherland 1998; Hawari et al. 2000a, 2000b; Beller and Tiemeier 2002; McCormick et al. 1981). In all three control columns RDX was not biodegraded at all. In these control columns ΔE_h between influent and effluent was between 100 and –150 mV. From these results, it seems the ultimate fate of RDX appears to be dependent on redox conditions. In treatment column systems, with ΔE_h between influent and effluent between -600 and -850 mV, RDX was transformed into nitroso- and non-nitroso-substituted metabolites. In Column T-T2 where ΔE_h between influent and effluent was the lowest (-850 mV) none of the nitroso-substituted transformation products was observed in the effluent stream. This might be because these nitroso-substituted intermediates are unstable at low redox and further undergo ring cleavage as postulated by other researchers (Hawari et al. 2000a, 2000b; McCormick et al. 1981). Oh et al. (2001) have tentatively identified a soluble intermediate MDNA as a result of ring cleavage. However, the formation and stability of MDNA as a biotransformation product of RDX under anaerobic conditions are not yet clear; it can occur as a transient intermediate (Halasz et al. 2002) or a stable transformation product (Oh et al. 2001).

In all three treatment columns, very little (~ 1 percent) of the inlet acetate concentration (500 mg/L as carbon) was used in the biological activity (Figure 8). Low (30-50 mg/L) levels of carbonate were observed in the effluent streams from these treatment columns.

RDX biodegradation kinetics

The rate of transformation of RDX in individual columns, under each temperature condition, was evaluated by fitting the advection-dispersion transport model with the contaminant decay model (Equation 2) to the axial RDX concentration profile along the column length. Two bed profile samplings were carried out at three different temperatures (15, 10, and 5 °C) to determine the average rate of RDX biotransformation with time of operation. Each temperature test lasted for 30 days, and bed profile samples were collected from intermediate ports along the column length at days 23 and 30.

Over time, the two concentration profiles did not vary for the individual columns; however, Column T-T2 behaved differently from the other two treatment columns. The presence of acetate as a carbon source (electron donor) resulted in the transformation of RDX into different nitroso-substituted products in the treatment columns. In the control columns (where no acetate was added) no biotransformation of RDX was observed throughout the column length. In all the bed profile tests performed at various operating temperatures, the predominant transformation product identified at intermediate ports in Column T-T2 was MNX, but in Columns T-T1 and T-T3 a sequential biotransformation of RDX into MNX, DNX, and TNX was observed. This pattern of transformation products may be a result of presence of different

microbial consortia because Column T-T2 was more reduced than Columns T-T1 and T-T3, which might have changed the microbial dynamics. Kitts et al. (1994) observed the similar variable microbial ability to transform RDX. The researchers reported that two species (*Morganella morganii* and *Providencia rettgeri*) completely transformed RDX and subsequent nitroso-substituted intermediates, and a third one (*Citrobacter freundii*) partially transformed RDX and generated high concentrations of nitroso-substituted intermediates. Bed profile analysis at individual operating temperature is described in detail in the following paragraphs.

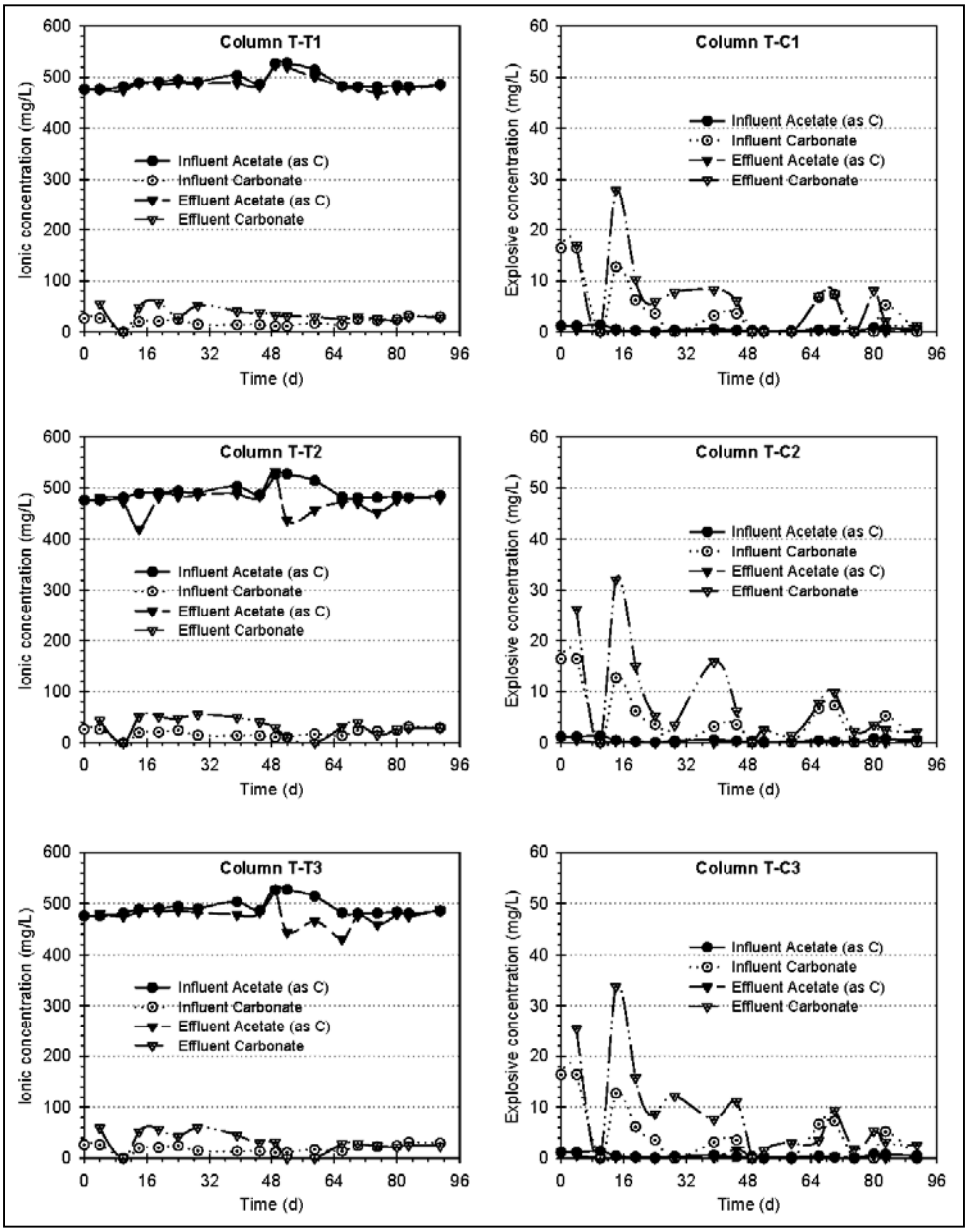

Figure 8. Amendment concentration in influent and effluent streams

Axial RDX and its nitroso-transformation product concentration profiles during two bed profile tests carried out at 15 °C are shown in Figures 9 and 10.

There was no significant difference between the two bed profile analyses for the individual columns. In Columns T-T1 and T-T3 the three nitroso-substituted metabolites were observed in a typical sequential manner with MNX followed by DNX and then TNX. However, in both the bed profile tests very low levels of MNX, and seldom DNX and TNX, were observed in Column T-T2. Furthermore these transformation products were very short lived because of the very reduced conditions (ΔE_h = -850 mV) in Column T-T2. In the control columns, no biotransformation of RDX was observed along the column length because of the lack of a reduced environment. In both bed profile tests, less than 1 percent of influent acetate concentration (about 500 mg/L as carbon) was used by the biological activity in the treatment columns (Figure 11). Very low (~50 mg/L) levels of carbonate were observed at intermediate ports in treatment columns.

Figure 12 illustrates the RDX biodegradation kinetic data for treatment columns at 15 °C. The advection-dispersion transport model with contaminant decay given in Equation 2 fitted very well to RDX concentration data from both bed profile tests. The first-order degradation rate coefficient *k* for RDX varied between 0.1297 and 0.1864 1/hr for the three treatment columns, with an average *k* value of 0.155 1/hr (standard deviation of 0.019). At this average *k* value the time needed for 50 percent removal of RDX is approximately 4.5 hr. RDX biodegradation kinetic parameters for individual columns are summarized in Table 2.

Table 2
RDX Biodegradation Rate Kinetics at Different Temperatures

Treatment Column	First–order Biodegradation Rate Coefficient *k* (1/hr) at Temperature					
	15 °C		10 °C		5 °C	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
T-T1	0.1455	0.1864	0.1242	0.1022	0.0604	0.0775
T-T2	0.1632	0.1548	0.0995	0.1017	0.0679	0.0769
T-T3	0.1297	0.1511	0.0896	0.0721	0.0422	0.0424
Average	0.155 (±0.019)		0.098 (±0.017)		0.061 (±0.016)	

Note: Average represents the mean of two tests for all three treatment columns at a particulate temperature. Values in parentheses are the standard deviation (n = 6).

Bed profile tests conducted at 10 °C are shown in Figures 13 and 14. There was no noticeable difference in the axial concentration of RDX and its nitroso-substituted transformation products in treatment columns. Similar to 15 °C tests, in Columns T-T1 and T-T3 a typical sequential transformation of RDX into MNX, DNX, and TNX was observed. Contrary to the 15 °C test, measurable levels of RDX were also observed in the effluent stream of these two columns. Furthermore, in both the bed profile tests the levels of these nitroso-substituted transformation products were higher than those found at 15 °C. In Column T-T2, although no RDX or nitroso-substituted metabolites were observed in the effluent stream, RDX degradation was considerably delayed along the column height. These results indicate the adverse effect of lower temperature on biological

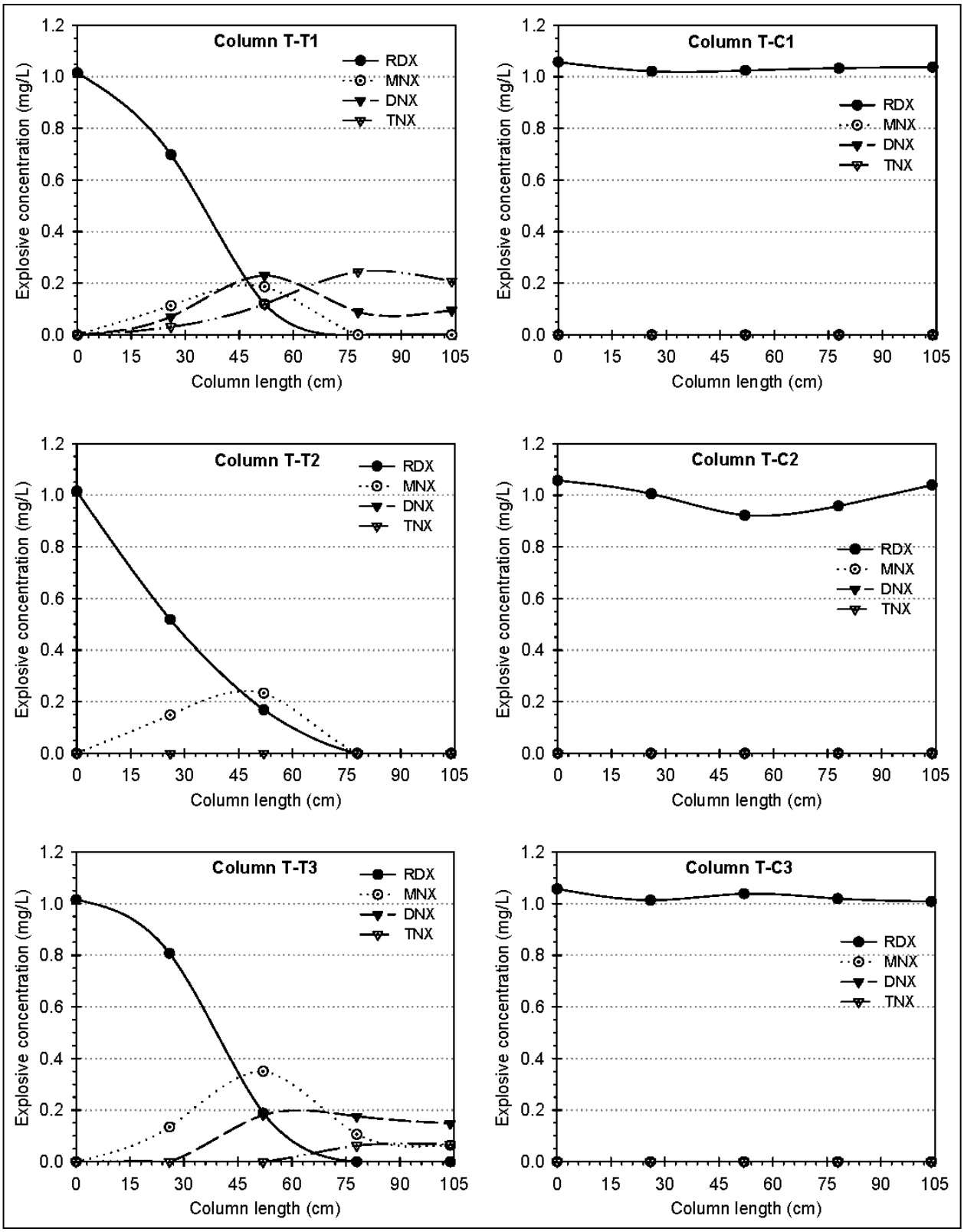

Figure 9. Axial concentration profile of RDX and its nitroso-substituted metabolites at 15°C (Test 1)

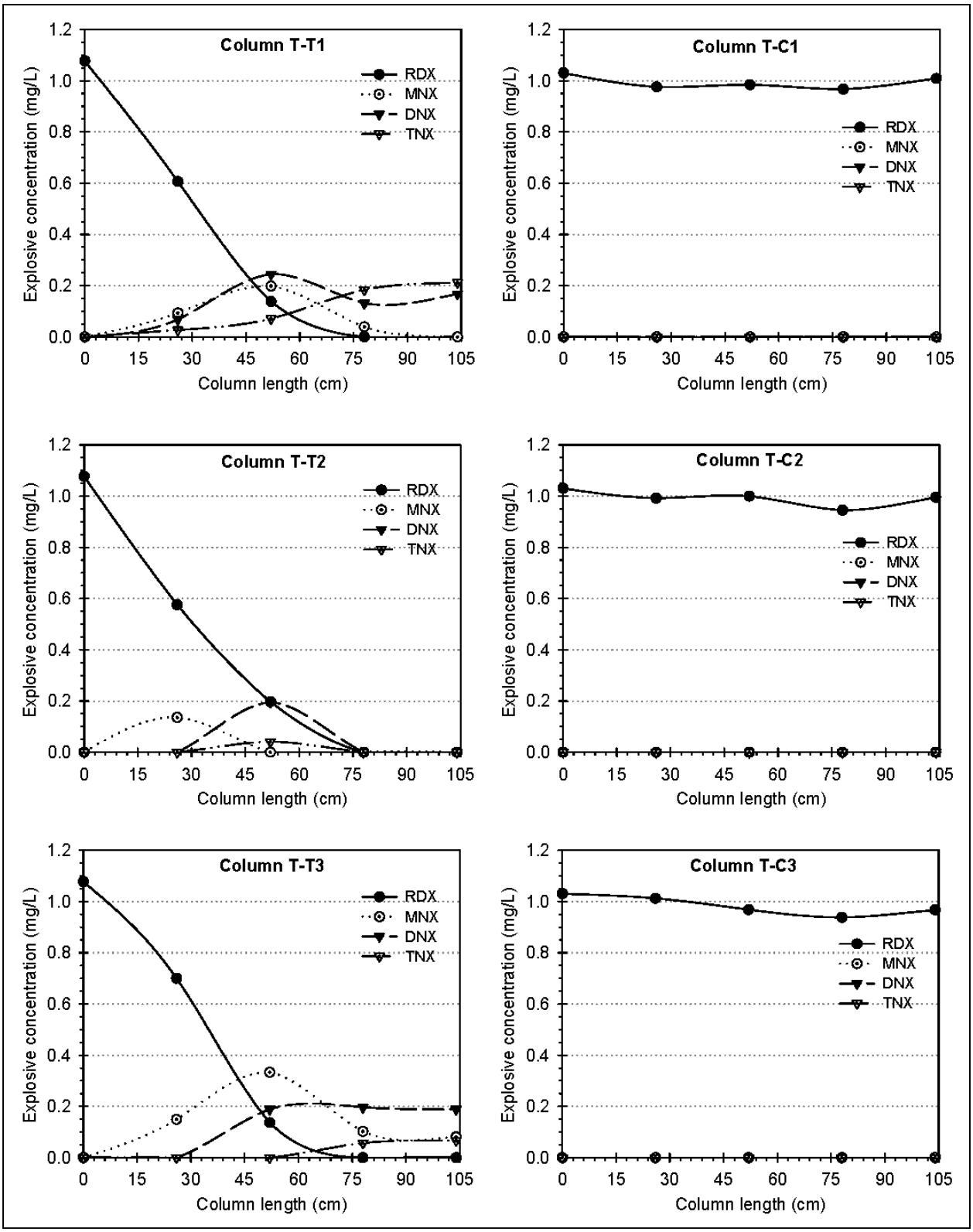

Figure 10. Axial concentration profile of RDX and its nitroso-substituted metabolites at 15°C (Test 2)

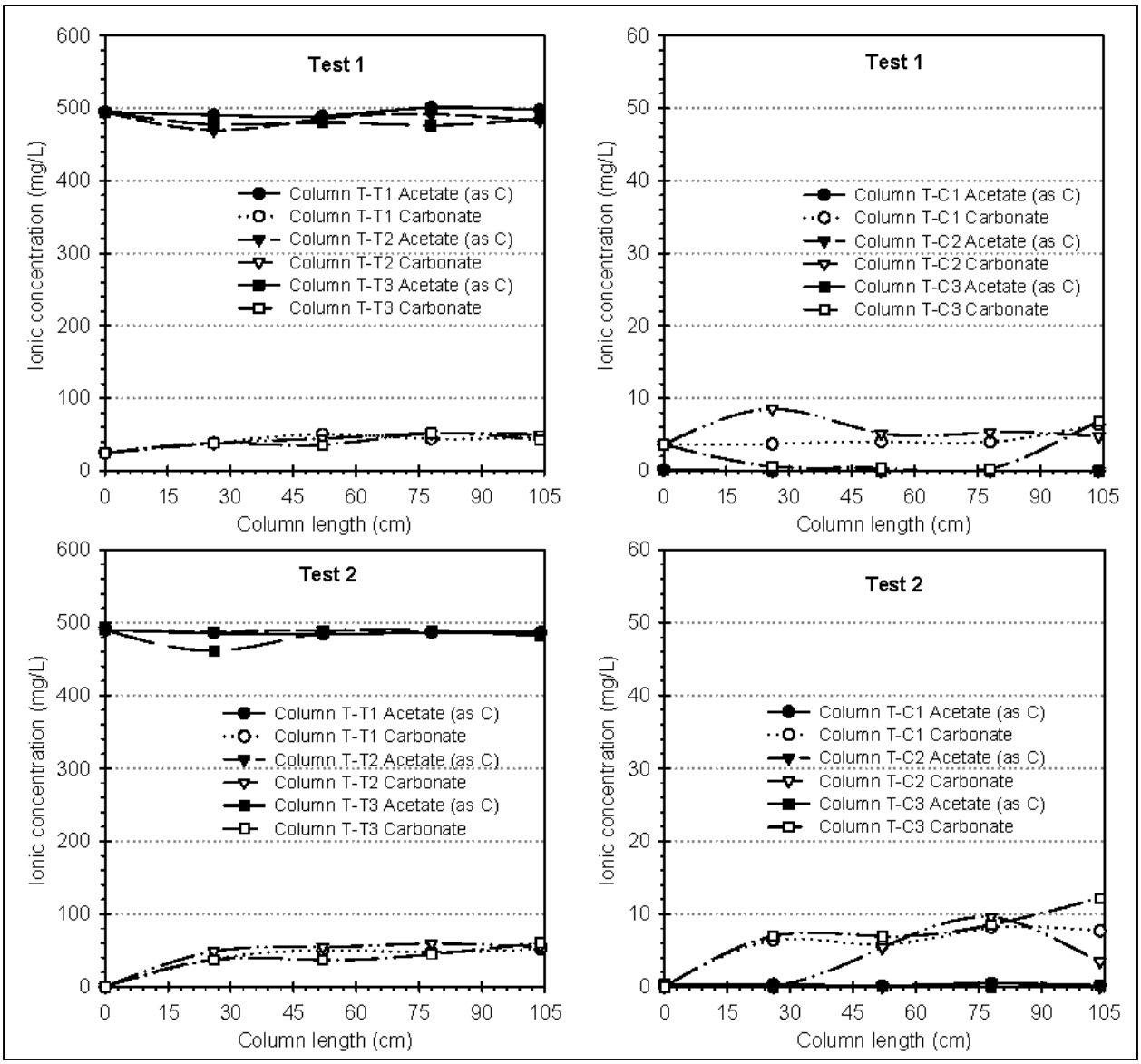

Figure 11. Axial concentration profile of acetate and carbonate at 15°C (Tests 1 and 2)

activity responsible for RDX biotransformation. No biotransformation of RDX was observed in either of the control columns because of lack of reduced conditions. In both bed profile tests, very little influent acetate (~500 mg/L as carbon) was utilized by the biological activity in the treatment columns (Figure 15). Significantly low (~50 mg/L) levels of carbonate were observed at intermediate ports in treatment columns.

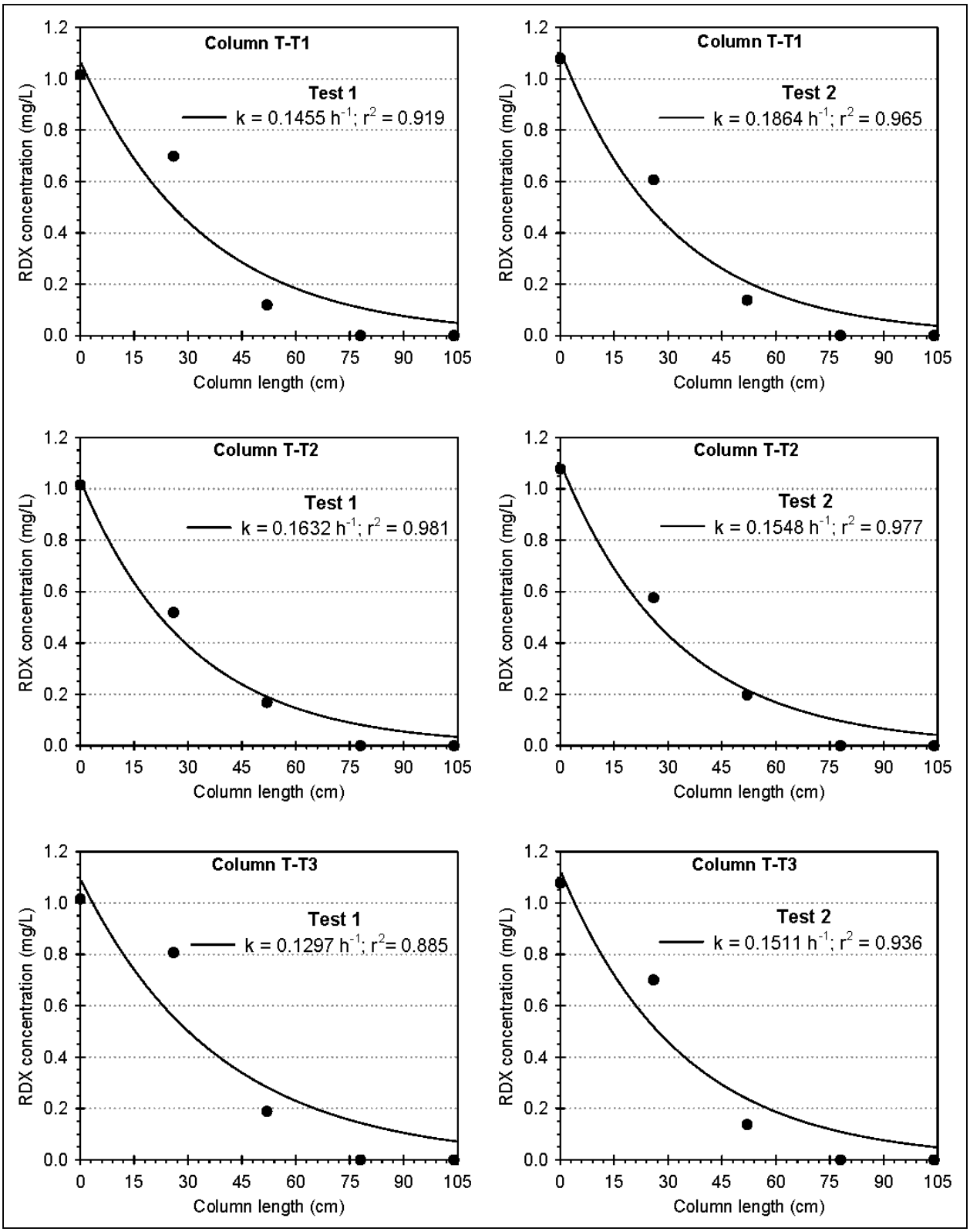

Figure 12. RDX biodegradation kinetic analysis in treatment columns at 15 °C (Tests 1 and 2)

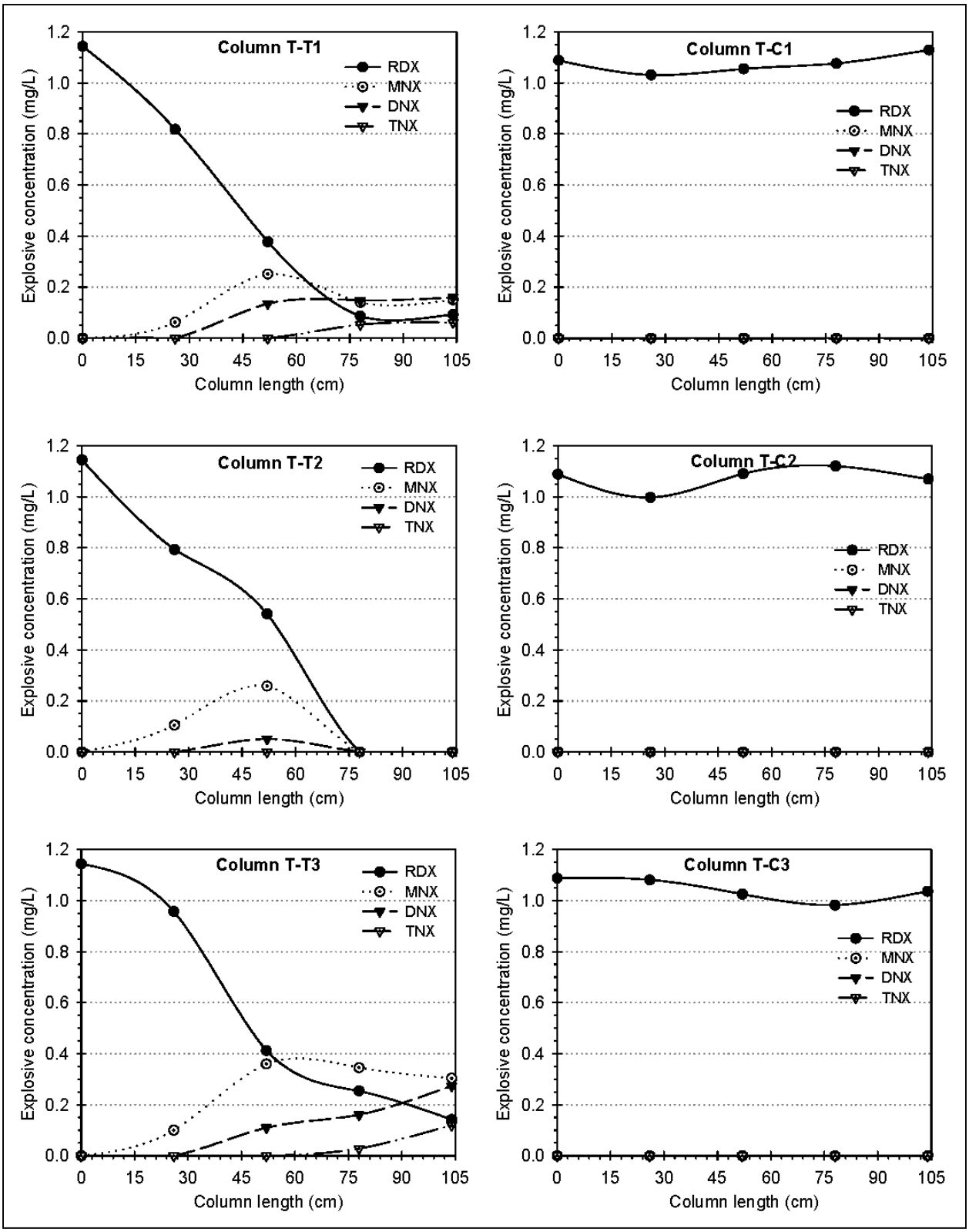

Figure 13. Axial concentration profile of RDX and its nitroso-substituted metabolites at 10°C (Test 1)

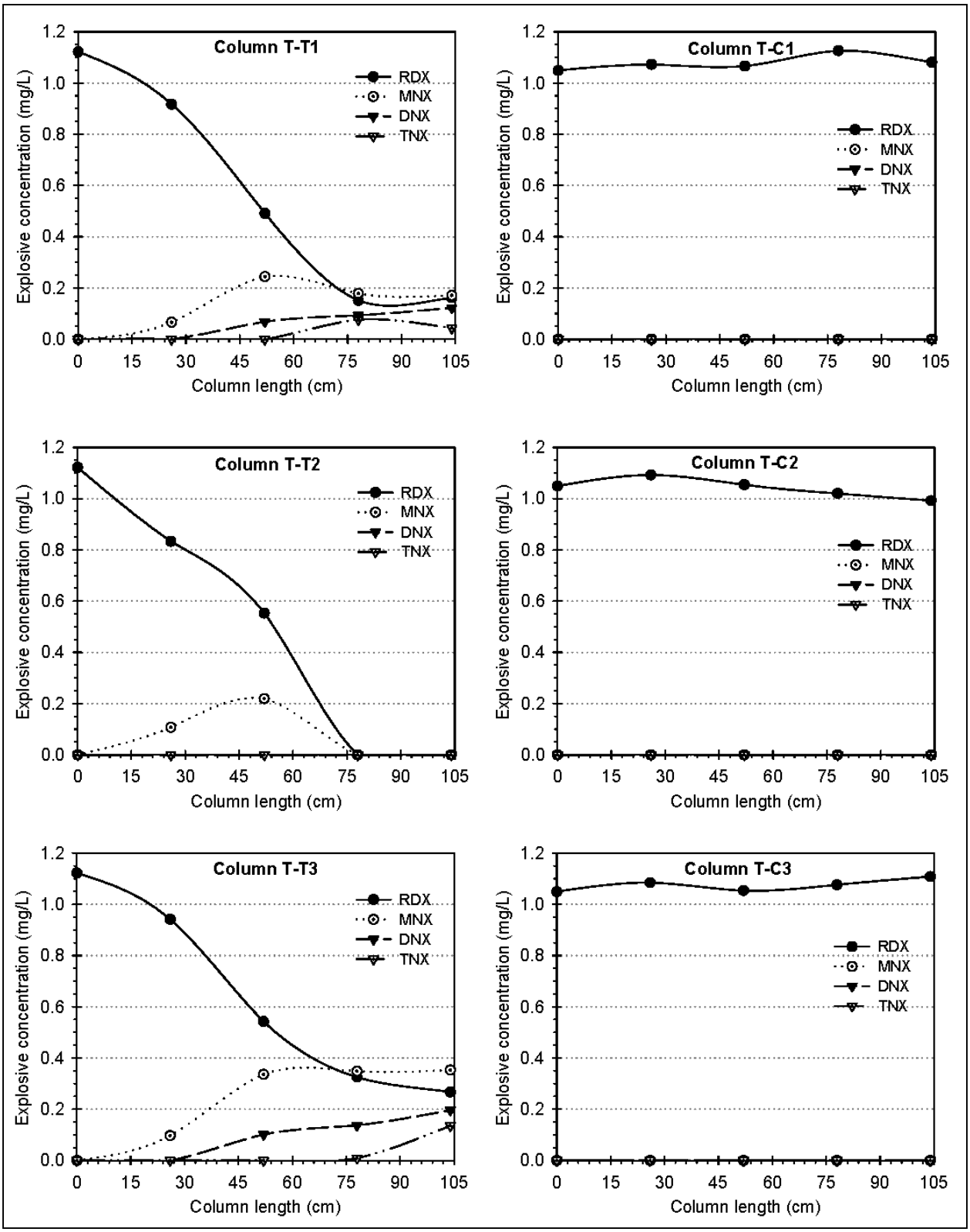

Figure 14. Axial concentration profile of RDX and its nitroso-substituted metabolites at 10°C (Test 2)

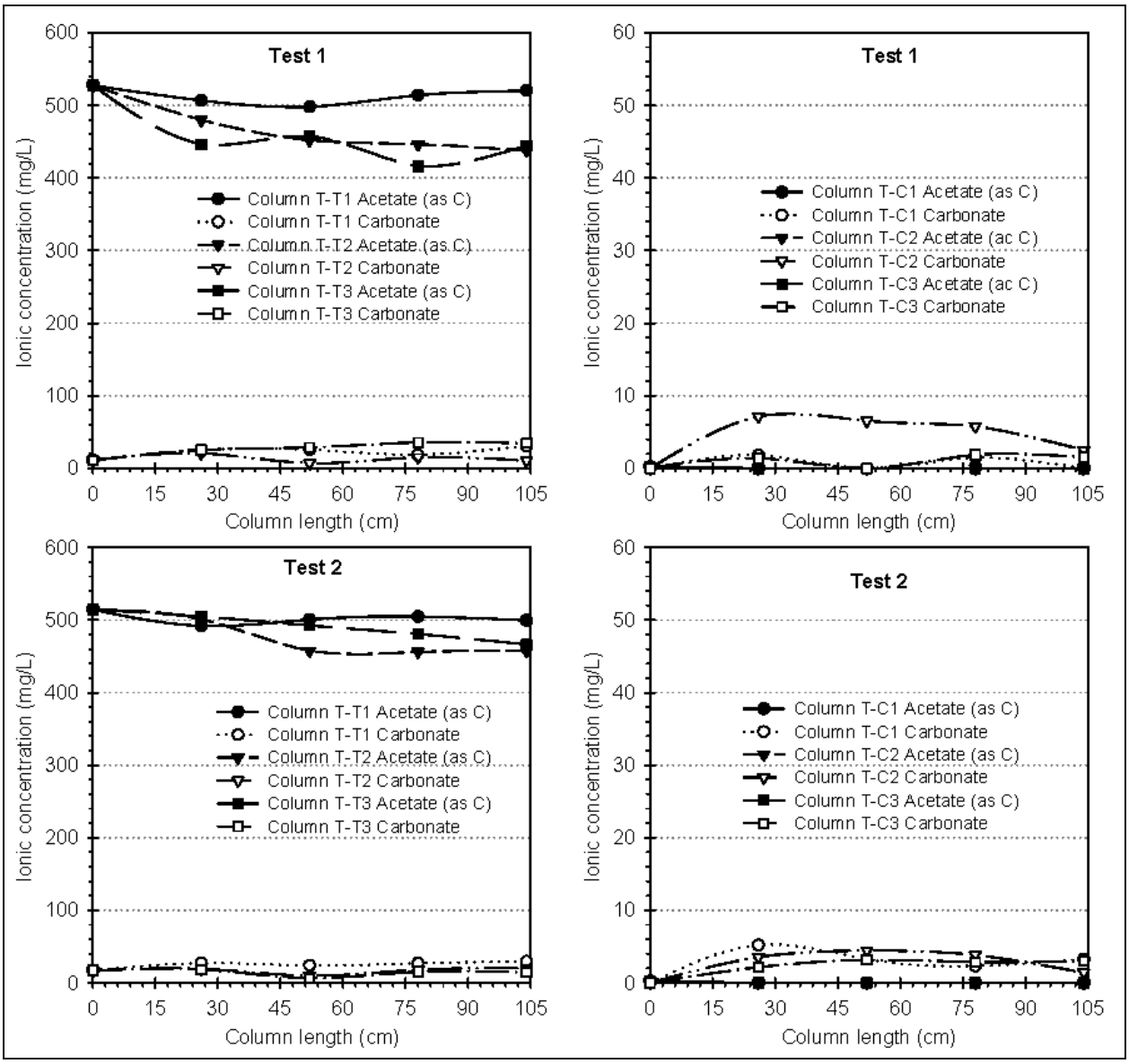

Figure 15. Axial concentration profile of acetate and carbonate at 10°C (Tests 1 and 2)

RDX biodegradation kinetic data for treatment columns at 10 °C are shown in Figure 16. Equation 2 fitted very well to the axial RDX concentrations from both the bed profile tests for all three treatment columns. The first-order biodegradation rate coefficient *k* values for RDX were significantly lower than those for 15 °C, and varied between 0.0721 and 0.1242 1/hr for the three treatment columns (Table 2). At the average *k* value of 0.098 1/hr (standard deviation of 0.017), time needed for the removal of half of influent RDX concentration is approximately 7 hr, roughly 50 percent longer than the time needed for the same percent removal at 15 °C. These results quantitatively demonstrate the adverse effects of lower aquifer temperature on biological activity and eventual RDX biotransformation rate.

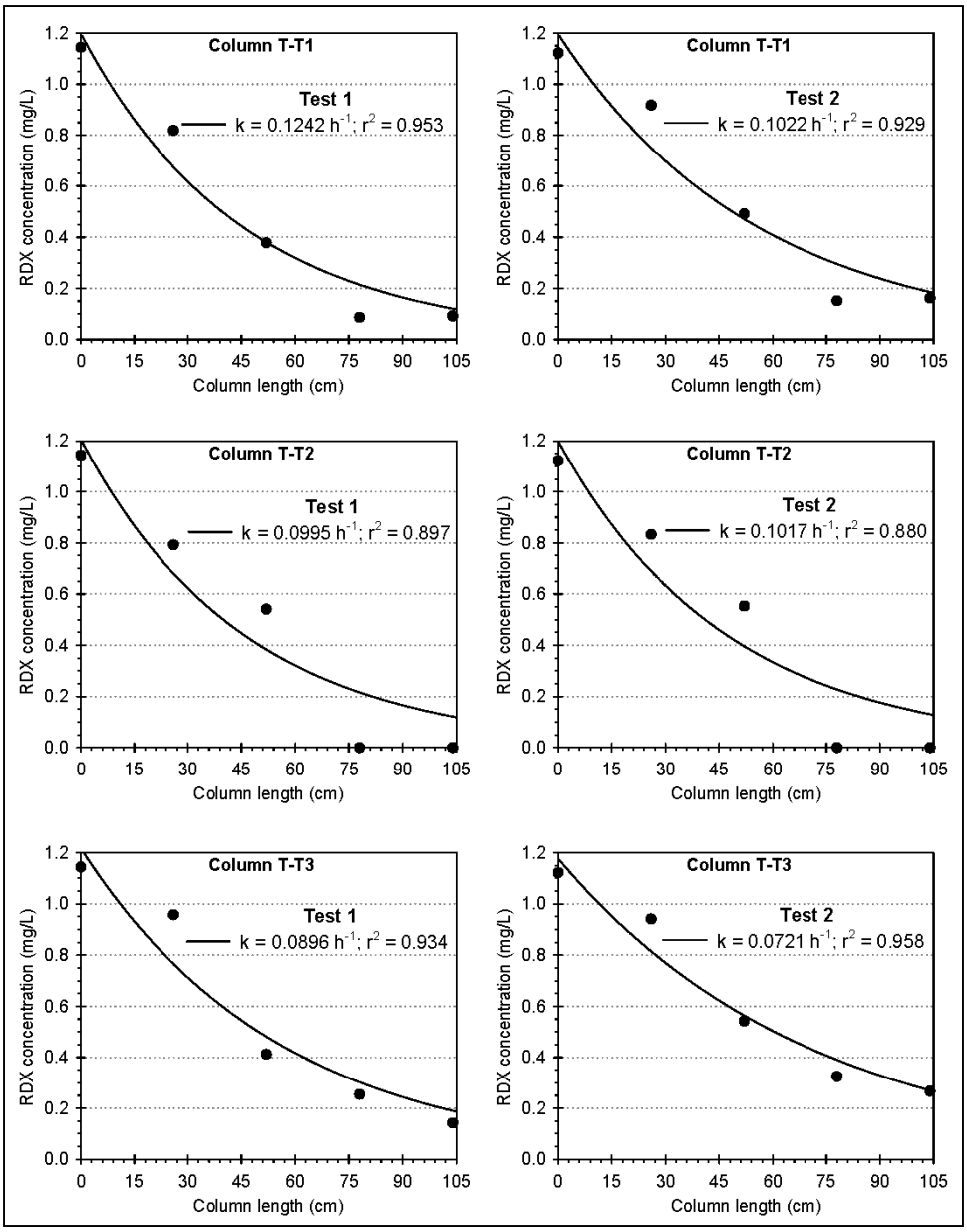

Figure 16. RDX biodegradation kinetic analysis in treatment columns at 10 °C (Tests 1 and 2)

At 5 °C two bed profile tests were performed. The results of axial concentrations of RDX, MNX, DNX, and TNX in treatment and control columns are shown in Figures 17 and 18. Explosives concentration profiles did not show any noticeable differences between the two bed profile analyses. Unlike the previous two tests conducted at 15 and 10 °C, low concentrations of RDX and MNX were observed in the effluent stream of each column during the 5 °C test. Concentrations were generally lower in Column T-T2. Additionally, in Columns T-T1 and T-T3 measurable concentrations of DNX and TNX were found in the effluent stream. No DNX or TNX was observed in Column T-T2, and the transient concentrations of MNX at intermediate sampling ports were not present in the column effluent. As discussed previously, the different behavior of

Column T-T2 resulted primarily from the very reduced conditions (ΔE_h = -850 mV) in this column compared with Columns T-T1 and T-T3. In the control columns, no biotransformation of RDX was observed along the entire column length because of lack of reduced conditions. In both bed profile tests, little of the influent acetate (~500 mg/L as carbon) was utilized by the biological activity in the treatment columns (Figure 19). Very low (~30 mg/L) levels of carbonate were observed at intermediate ports in treatment columns.

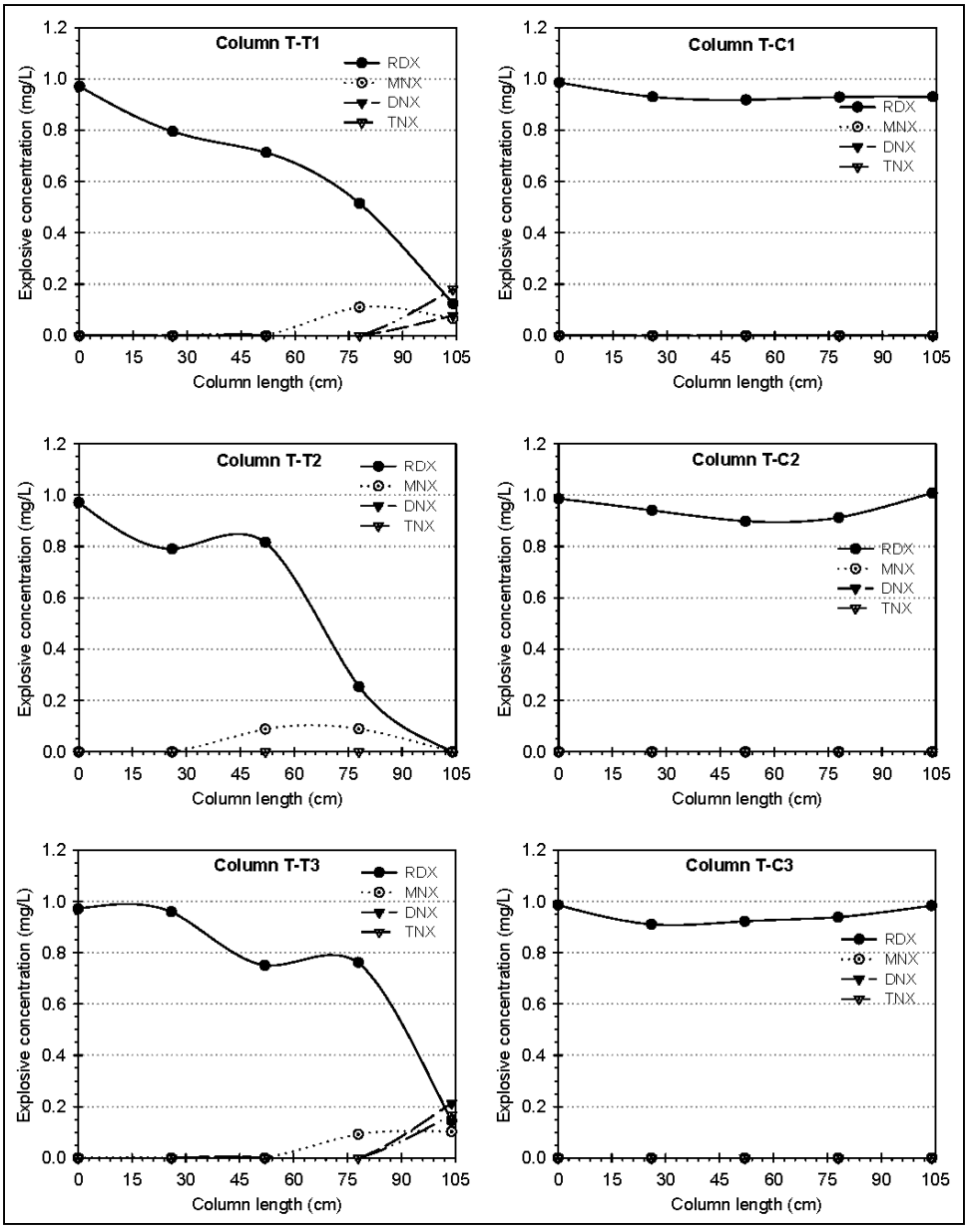

Figure 17. Axial concentration profile of RDX and its nitroso-substituted metabolites at 5°C (Test 1)

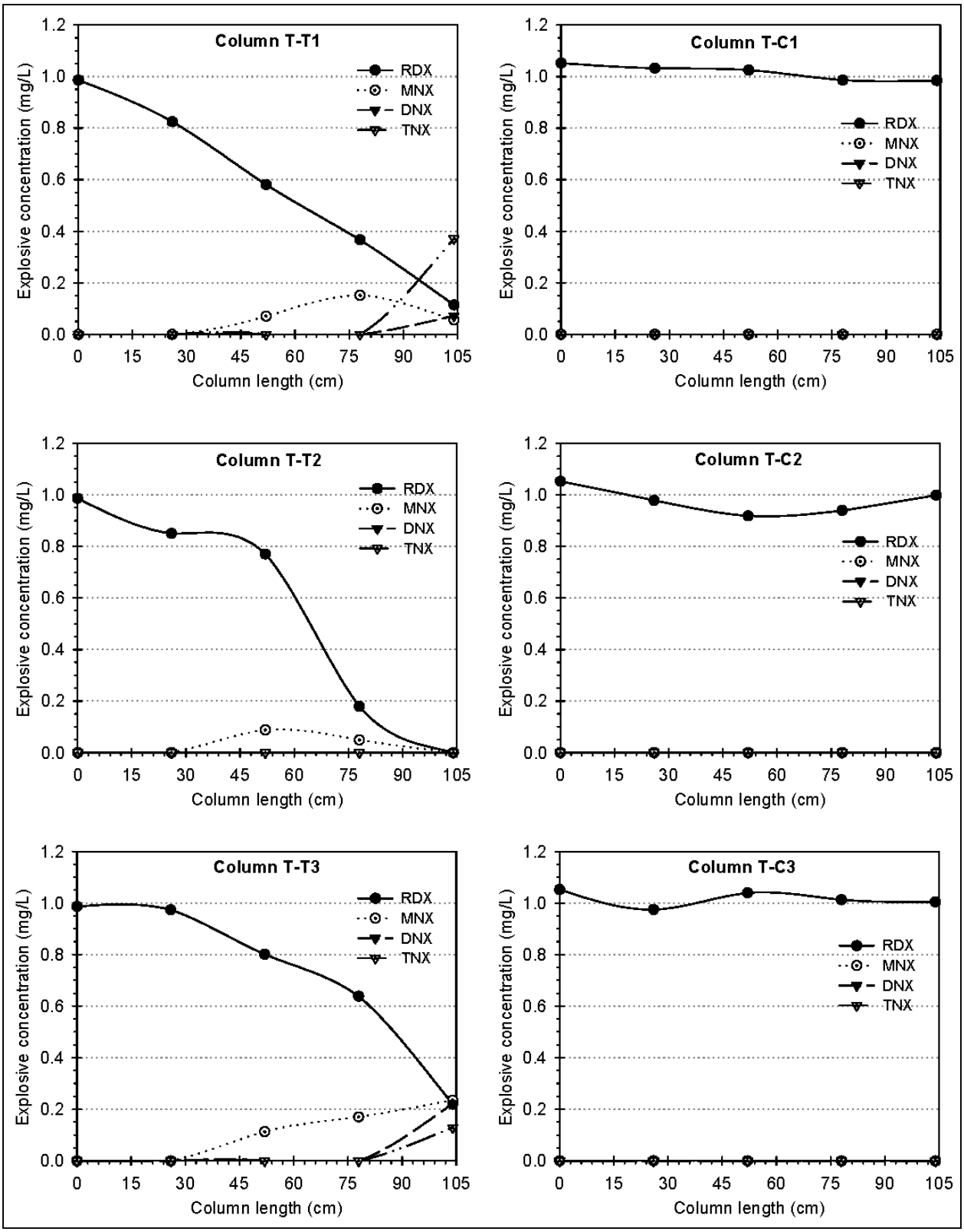

Figure 18. Axial concentration profile of RDX and its nitroso-substituted metabolites at 5°C (Test 2)

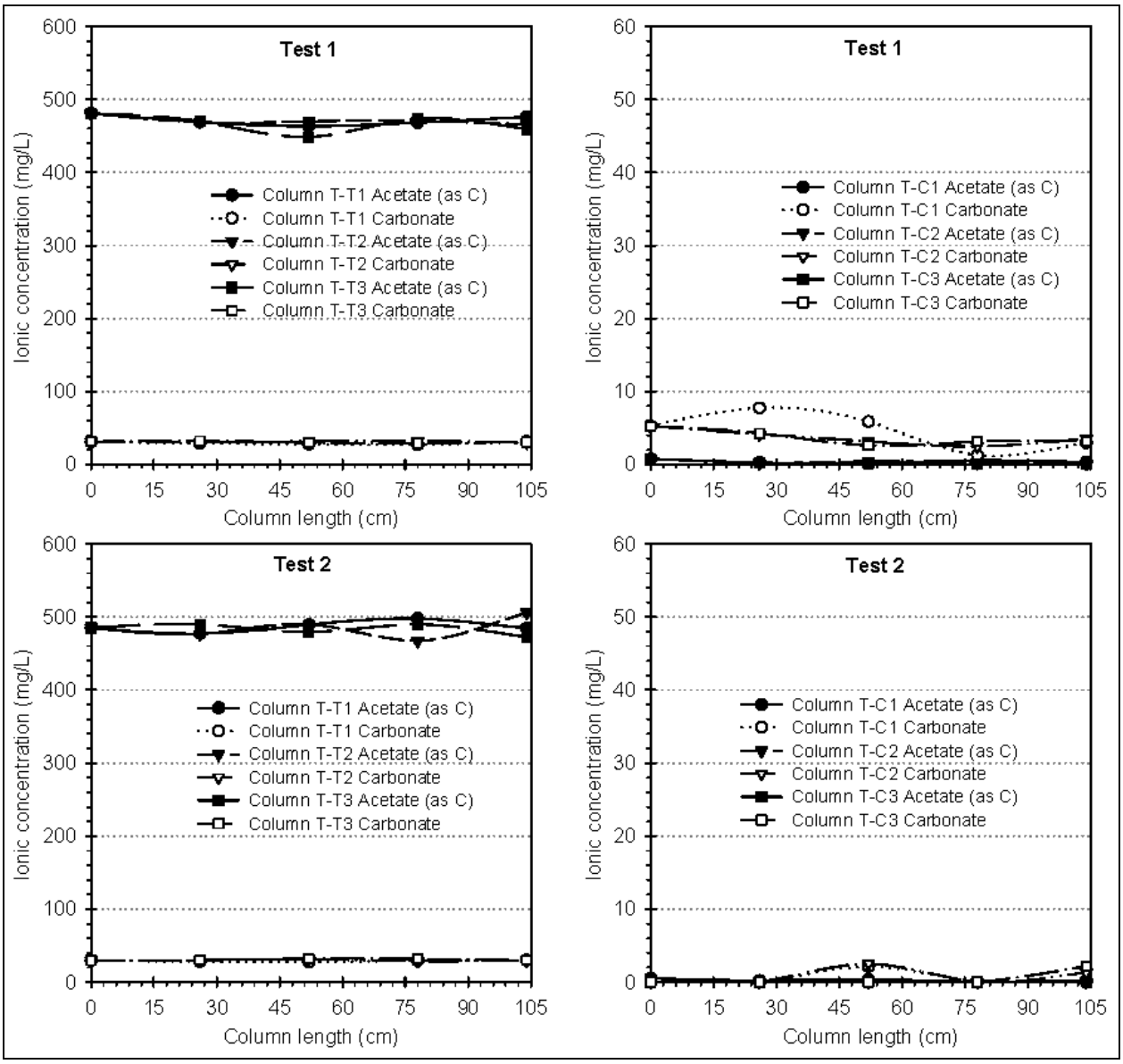

Figure 19. Axial concentration profile of acetate and carbonate at 5°C (Tests 1 and 2)

Figure 20 illustrates the RDX biodegradation kinetic analysis at 5 °C. The first-order degradation rate coefficient k for RDX varied between 0.0422 and 0.0775 1/hr (Table 2) for the three treatment columns, with an average *k* value of 0.061 1/hr (standard deviation of 0.016). These *k* values are significantly lower than the *k* values obtained at 15 and 10 °C. The estimated time needed for biodegradation of half of the influent RDX concentration at this average *k* value is approximately 11.3 hr.

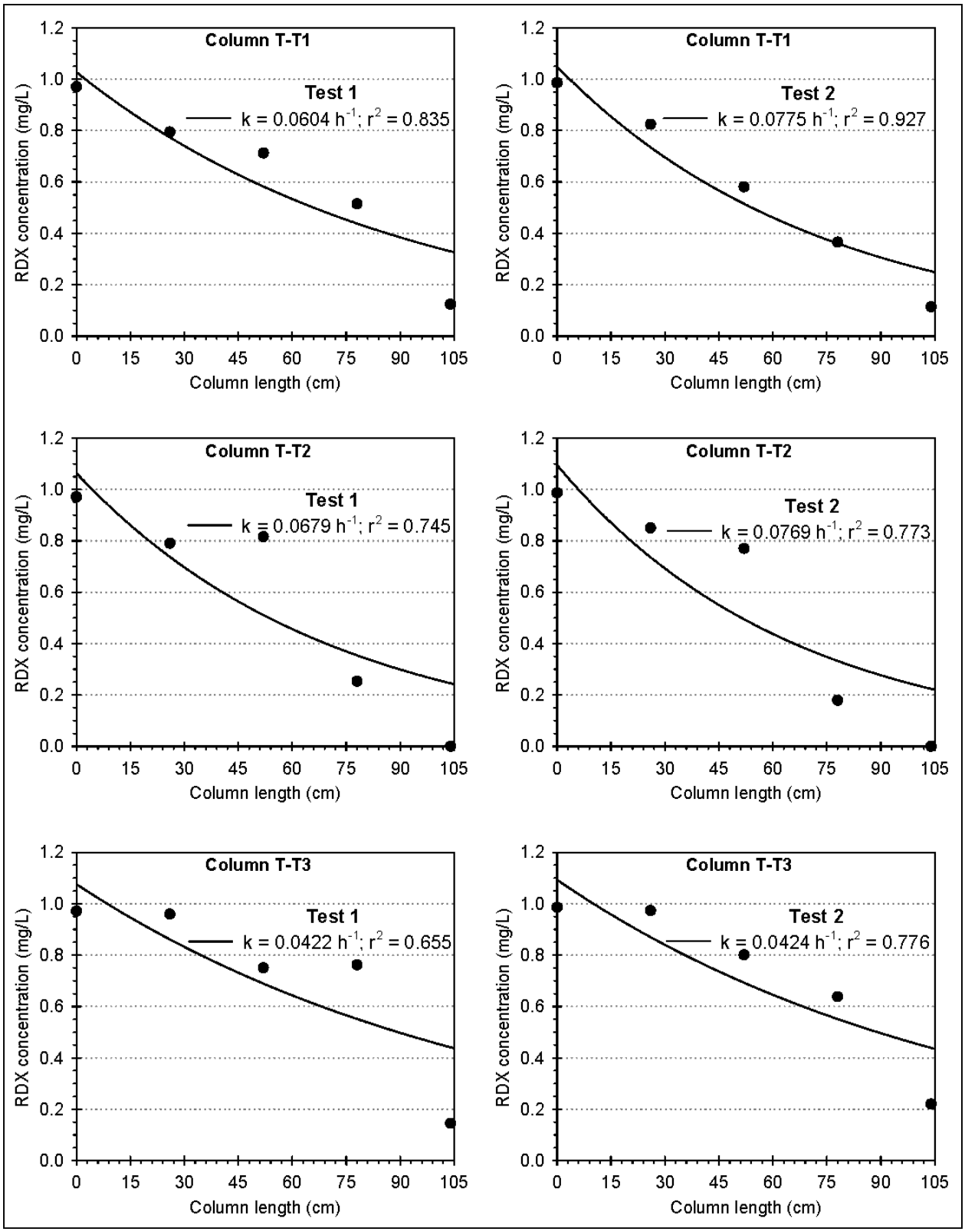

Figure 20. RDX biodegradation kinetic analysis in treatment columns at 5 °C (Tests 1 and 2)

The estimated *k* values at three different temperatures were significantly different (95 percent confidence) from each other. Statistical analysis was done by using the Tukey Test for pairwise multiple comparisons. The results of One Way Analysis of Variance showed that the differences in the mean values of *k* (n = 6) obtained at 5, 10, and 15 °C are statistically significant ($P < 0.05$).

The influence of aquifer temperature on RDX biotransformation was estimated by fitting the Arrhenius model (Equation 3) to the average *k* values obtained at different temperatures. Figure 21 summarizes the relation between operating temperature and the estimated first-order biodegradation rate coefficients for RDX in treatment columns. As evident from Figure 21, aquifer temperature has a significant influence on the in situ biodegradation of RDX. For these experimental conditions, an activation energy of about 63.54 kJ/mol of RDX was estimated.

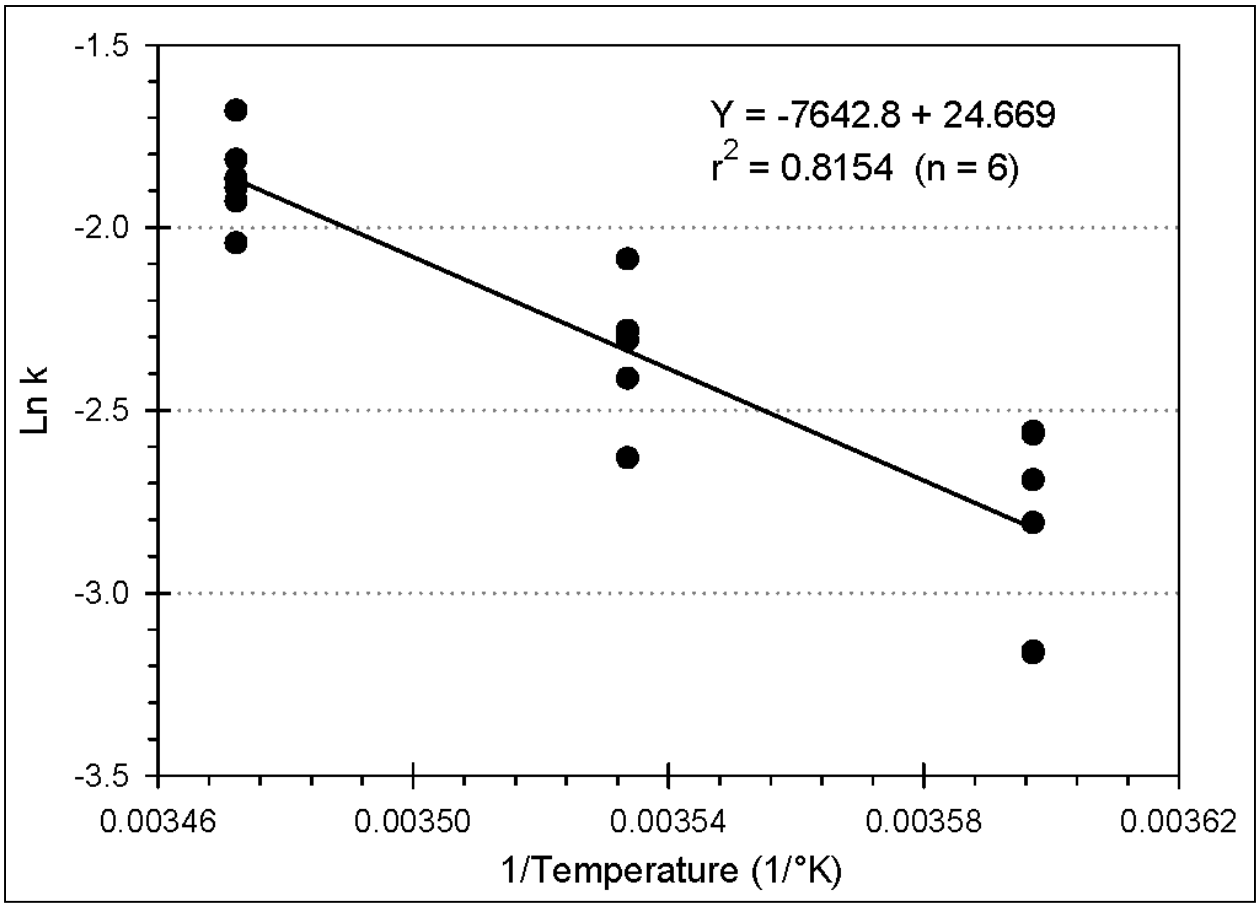

Figure 21. Temperature influence on RDX biotransformation kinetics

Radiolabel Study

Column hydrodynamics

RDX-contaminated water flow in both triplicate column sets during the 9-week study was around 0.2 mL/min, equivalent to a liquid velocity of 0.85 m/day (2.7 ft/day) (Figure 22). This water flow resulted in liquid residence time of approximately 24 hr in individual columns. Due to equipment breakdown only two columns were used for amendment treatment and control. The slug of radiolabel RDX (~ 0.76 μCi) was introduced on day 51. After that 10 bed volumes of unlabeled RDX-contaminated groundwater were pumped through each column over the next 10 days to wash out any radioactivity sorbed on the aquifer material. As shown in Figure 22, groundwater flow rate during this time was slightly higher than 0.2 mL/min.

Anaerobic conditions were established in treatment columns by providing a carbon source to indigenous microorganisms, which then utilized oxygen, creating a reduced environment. In treatment columns, E_h drop was significant, ranging between -550 and -700 mV (Figure 22). The drop in redox potential was more significant in Column R-T2 (-700 mV) than in Column R-T1 (-550 mV). One possible reason may be a higher concentration of RDX degrading microorganisms that utilized oxygen in the presence of a readily available carbon source, creating a very reduced environment. This explanation of higher biomass was also evident from the back pressure data (Figure 22), which was highest in Column R-T2 probably due to biofouling. Since no carbon source was used in the control columns, the drop in redox potential was very small (between 70 and -70 mV) compared with those of the treatment columns.

Influent stream pH varied between 7 and 7.5 for treatment columns where RDX-contaminated water was amended with acetate as the electron donor (Figure 22). In the control columns, influent water pH was slightly higher, between 8 and 8.5. The effluent from treatment columns showed a slight increase in pH (8 to 8.5); however, in the control columns there was a slight decrease in effluent pH (6.5 to 7). The effluent from both treatment and control columns during the actual radiocarbon test (final 2 weeks of the study) was collected in an acid quencher (containing 1N HCl) to prevent the degradation of any untreated RDX in the effluent stream at high pH, and also to release any dissolved mineralization-carbon dioxide from the effluent stream. The effluent stream pH (1.5 to 2) during the final 2 weeks of the test shown in Figure 22 actually is not the effluent stream pH but rather the pH of the contents in the acid quencher.

There was no significant back pressure buildup due to biofouling in any of the columns except Column R-T2 where head loss increased steadily and remained around 70 kPa (10 psi) during the last 2 weeks (Figure 22). This increased back pressure could be the result of a higher biomass yield that coincided with the highest drop in redox potential in Column R-T2 because of increased biological activity consuming oxygen in the presence of the electron donor. Occasional hikes in the back pressure for Column R-C1 were due mainly to plugging of the porous PVC screen at the column inlets.

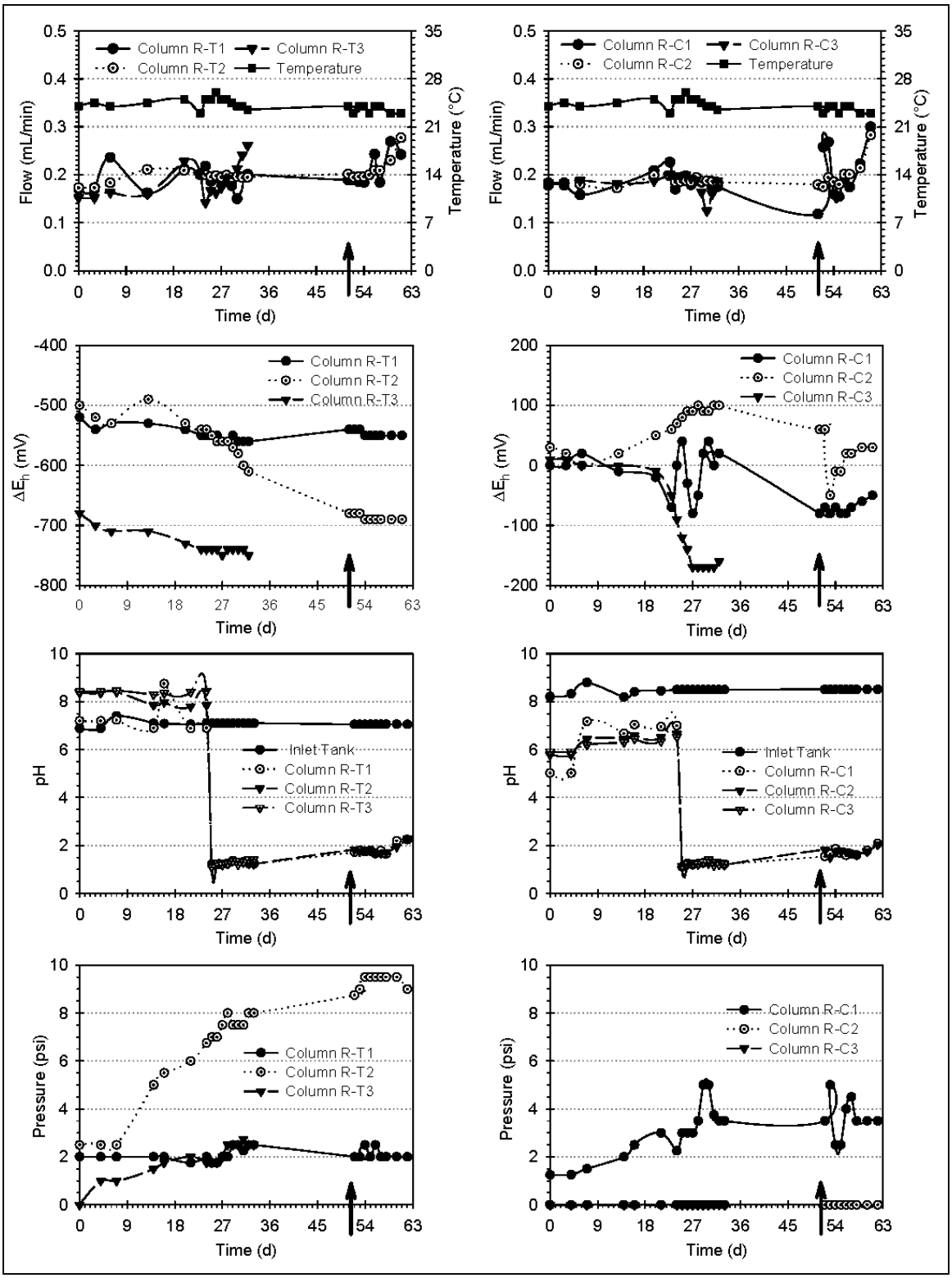

Figure 22. Feed water flow, change in redox, pH, and backpressure in radiolabel columns (note: to convert pressure to kilopascals, multiply by 6.9)

RDX biotransformation

RDX concentrations (around 1 mg/L) in the influent groundwater were reduced to below detection limits of 0.02 mg/L in Column R-T2 without the detection of any nitroso-substituted RDX derivatives. However, in Column R-T1 low concentrations of RDX, MNX, and DNX were observed in the effluent stream during acclimation stages, i.e., while the reductive environment was developing in the column (Figure 23). During the actual radiolabel test (final 2 weeks of the study) as the redox decreased, only low concentrations of RDX and MNX were observed in the effluent stream. In Column R-T2 the redox was very low compared with Column R-T1, which may explain the RDX biodegradation without the detection of any nitroso metabolites. Column R-T2 also exhibited a steady increase in back pressure (Figure 22). One plausible reason behind these two manifest observations in Column R-T2 could be a higher biomass yield that caused RDX biodegradation without the detection of any nitroso transformation products and at the same time created a higher flow resistance resulting in higher back pressure along the column length. The assumption of high biomass yield is also substantiated by the lowest redox potential in Column R-T2 as a result of higher biological activity. The cumulative presence of untreated RDX and nitroso-substituted RDX metabolites in Column R-T1 accounted for about 20 percent of the influent RDX concentration. The unaccounted 80 percent of the inlet RDX might include volatile (including mineralized carbon dioxide) and nonvolatile non-nitroso-transformation products as proposed by other researchers (Hawari et al. 2000a, 2000b; McCormick et al. 1981). In Column R-T2, entire initial RDX concentration was transformed into volatile and nonvolatile non-nitroso-substituted transformation products.

In control columns very little biodegradation of RDX was observed throughout the course of study (Figure 23). Especially during the last 2 weeks of the study, when radiolabel was introduced, about 8-10 percent of the initial RDX concentration was biodegraded/transformed into products other than nonvolatile nitroso-substituted derivatives, because MNX. DNX, or TNX was not observed in the effluent stream.

During the 9-week study, RDX was removed from the groundwater and low levels of nitroso-substituted transformation products were detected in the treatment Column R-T1; however, in treatment Column R-T2 effluent none of the nitroso-derivates was observed. This variation in RDX end products within these two treatment columns was mainly redox dependent. In Column R-T1, ΔE_h between influent and effluent stream was around –550, whereas ΔE_h was very low (-700 mV) in Column R-T2 where none of the nitroso-substituted transformation products was observed in the effluent stream. In control columns very little RDX was biodegraded. In these control columns ΔE_h between influent and effluent was between 50 and -60 mV. From these results, it appears that two pathways that are highly redox dependent may be present. One pathway is sequential reductive transformation of nitro functional groups to nitroso-derivatives (Figure 1) as reported for various RDX-metabolizing cultures that use organic electron donors (Freedman and Sutherland 1998; Hawari et al. 2000a; Beller and Tiemeier 2002; McCormick et al. 1981). Another pathway may be the

direct attack of the ring as proposed by Hawari et al. (2000b). This direct attack resulting in ring cleavage may be active only at low redox potentials. Similar results of non-nitroso-substituted reductive biotransformation of [^{14}C]RDX by aquifer microorganisms have been reported by Beller (2002). MDNA, a non-nitroso ring cleavage intermediate, has been recently identified by Oh et al. (2001) and Halasz et al. (2002).

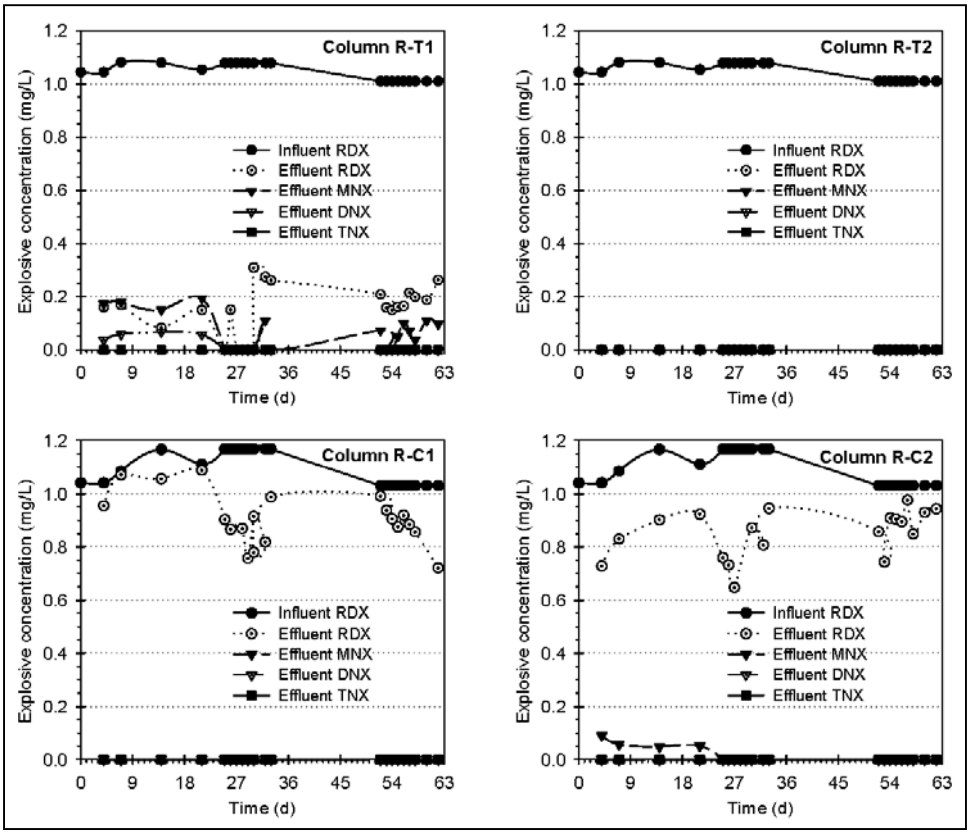

Figure 23. RDX and nitroso-derivatives concentration in influent and effluent from radiolabel columns

Radiocarbon (^{14}C) distribution

The distribution of radiocarbon (^{14}C) in treatment and control columns is summarized in Figure 24. In each column a slug of 0.77 µCi (1.7 million dpm) of radiolabel RDX was added in the inlet tank. The final mass balance on radiocarbon ranged between 76 and 87 percent in treatment columns, and more than 91 percent in control columns. The radiocarbon activity was distributed into three different carbon fractions: (a) dissolved (as aqueous soluble compounds), (b) mineralized (as carbon dioxide), and (c) anabolyzed (assimilated on biomass and/or sorbed on suspended material). The distribution of these three carbon fractions was different in treatment and control columns.

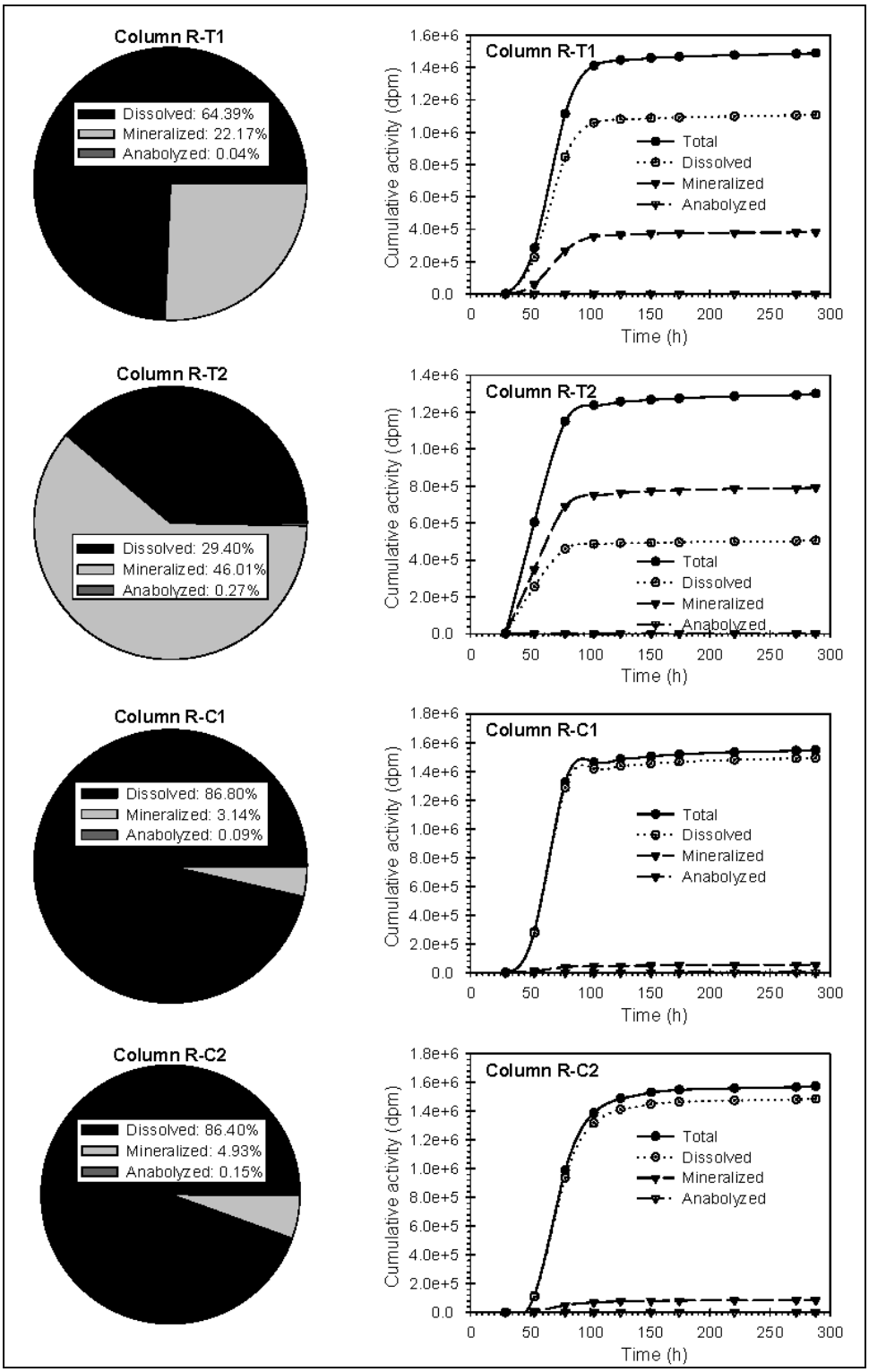

Figure 24. Distribution of ^{14}C activity from [^{14}C]RDX in radiolabel columns

The observed distribution of radiocarbon was quite different in the treatment columns. In Column R-T1 mass balance on ^{14}C accounted for 87 percent of initial activity, with approximately 65 percent in the dissolved fraction and 22 percent as mineralized carbon dioxide (Figure 23). The mass balance of radiocarbon in Column R-T2 accounted for about 76 percent of initial ^{14}C activity. In Column R-T2 the mineralized fraction (~46 percent) was much higher than the dissolved fraction (~30 percent). One plausible reason behind higher rate of mineralization in Column R-T2 may be higher concentration of biomass in this column that coincided with the higher back pressure because of biofouling as well as a higher drop in redox potential (Figure 22) as a result of higher utilization of oxygen by these RDX-degrading microorganisms. Even though a considerable amount of initial radiocarbon was mineralized to carbon dioxide by resident RDX-degrading microorganisms in both treatment columns, only a negligible amount was assimilated into biomass because the suspended fraction accounted for less than half a percent of initial activity (Figure 23).

Other researchers have measured mineralization of [^{14}C]RDX under reducing conditions with varying results. McCormick et al. (1981) recovered 1.5 percent of initial radiocarbon as $^{14}CO_2$ during anaerobic degradation of [^{14}C]RDX. Similar results, with <2 percent mineralization of radiolabel RDX, were reported by Beller (2002) using enrichment cultures with hydrogen as a sole electron donor. Kitts et al. (1994), studying three different bacterial species, recovered 5-9 percent of initial ^{14}C as $^{14}CO_2$ under anoxic conditions. Morley et al. (2002) recovered 8-30 percent of the initial [^{14}C]RDX as $^{14}CO_2$ in their batch experiments with ethanol and mixed carbon (mixture of glucose, glycerol, and succinate) as sole electron donors. An exceptionally high (60 percent) conversion of [^{14}C]RDX to $^{14}CO_2$ has been reported by Shen et al. (2000) in treating contaminated soil slurries using municipal anaerobic sludge. These studies demonstrate a wide range of mineralization potential of different microbial consortia using various carbon sources as electron donors.

The final mass balance closure in treatment columns indicates a failure to measure possible ^{14}C end products. Other researchers have reported similar problems in accounting for all the radiocarbon end products in their batch experiment where the final mass balance closure was only 79 percent of the initial [^{14}C]RDX (Morley et al. 2002). The unaccounted fraction of the initial ^{14}C activity in these treatment columns probably was converted to some products other than mineralized carbon dioxide and nonvolatile nitroso-metabolites. Previously Beller (2002) has reported that about 0.8 percent of [^{14}C]RDX was converted to volatile carbon other than carbon dioxide by enrichment cultures with hydrogen as the sole electron donor. However, in this study no attempt was made to identify these non-carbon dioxide volatile carbon compounds. In treatment columns, the dissolved fraction contained very low or undetectable concentrations of such nonvolatile nitroso-substitutes as MNX, DNX, or TNX. The RDX degraders (a mixed aquifer culture) present in the columns converted RDX to nonvolatile metabolites other than MNX, DNX, and TNX. Metabolites such as hydrazine, 1,1-dimethyl- and 1,2-dimethylhydrazine, MDNA, and formaldehyde that have previously been identified (Hawari et al. 2000a, 2000b; McCormick et al. 1981) with anaerobic RDX biodegradation may have been operationally included with nonvolatile carbon in this study. No specific analyses

were performed to identify these compounds, some of which are known to be unstable in aqueous solution.

In control columns, the majority (>86 percent) of the initial radiocarbon was in the dissolved phase, and very little (<5 percent) was mineralized. The fraction of ^{14}C in biomass and on suspended matter was negligible (Figure 23). Because of the lack of a carbon source, the redox potential in the control columns was not conducive to degradation of RDX. Also in the absence of a carbon source the biomass was not able to cometabolize RDX effectively. Distribution of ^{14}C over time, illustrated in Figure 23, shows a steady increase in the identified radiocarbon fractions over the first 100 hr in both treatment and control columns, which then stabilized over the next 200 hr without any significant increase.

6 Conclusions

The column study reported herein provides several elements of useful information on the fate of RDX during in situ reductive biotransformation in groundwater and the influence of aquifer temperature on the RDX biotransformation process. The temperature study showed that the rate of RDX biotransformation is adversely affected by the lower aquifer temperatures. In amendment treatment columns, with every 5 °C drop in operating temperature the RDX biodegradation rate coefficient was reduced by about 37 percent. The estimated first-order biodegradation rate coefficients for RDX at 15, 10 and 5°C were estimated to be 0.155, 0.098, and 0.061 1/hr, respectively. The activation energy, estimated from the temperature dependency of the rate coefficients evaluated using the Arrhenius model, was determined to be 63.54 kJ/mol.

The radiolabel study demonstrated that the fate of RDX subject to in situ biodegradation is highly dependent on redox conditions in the aquifer. In acetate-amended columns a considerable portion (23-46 percent) of initial radiocarbon was mineralized to $^{14}CO_2$, compared with <5 percent in amendment control columns. Moreover, the composition of the dissolved fraction was significantly different between amendment treatment and amendment control columns. In treatment columns, where the dissolved fraction of initial radiocarbon was estimated to be between 46 and 64 percent, no nitroso-substituted RDX transformation products were identified. In these treatment columns, where the drop in redox potential was between -550 and -700 mV, the nitroso-substituted intermediates were further degraded probably via cleavage of the triazine ring as reported by previous researchers (McCormick et al. 1981; Hawari et al. 2000a). In amendment control columns, where the reduction in redox potential was very low (70 to -70), the major portion of the dissolved fraction was RDX.

Based on the results of this study, it can be concluded that RDX can be substantially biotransformed under low redox conditions. Furthermore aquifer temperature has a significant influence on the rate of RDX biodegradation, and will therefore be a major factor in determining the length of the treatment zone in actual field applications. The necessary reduced conditions can be achieved by providing sufficient quantities of a readily biodegradable carbon source such as acetate to consume additional oxidants like oxygen and to exceed the demands for other ubiquitous inorganic electron acceptors such as nitrate and sulfate. Finally, to achieve the biodegradation of RDX and its nitroso derivatives, and to avoid the accumulation of toxic nitroso-substituted metabolites, a very low redox is mandatory.

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14. ABSTRACT

A series of column studies with site-specific aquifer material from the former Nebraska Ordnance Plant were performed to evaluate the influence of aquifer temperature on in situ hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) biodegradation, and to assess the ultimate fate of RDX in groundwater under biologically induced reductive conditions. In treatment columns RDX-contaminated water was amended with acetate as readily available carbon source, and in control columns no electron donor was used. The results of the temperature study demonstrated clear indications of adverse effects of lower aquifer temperature on biological activity of RDX-degraders. As the aquifer temperature decreased from 15 to 10 and eventually to 5 °C, the concentration of nitroso-substituted metabolites and untreated RDX increased in the effluent stream. The estimated first-order biodegradation rate coefficient *k* for RDX at 15 °C was 0.155 1/hr (±0.019, n = 3). This rate coefficient decreased by about 37 percent to 0.098 1/hr (±0.017, n = 3) at 10 °C, and by another 38 percent to 0.061 1/hr (±0.016, n = 3) at 5 °C. An activation energy of 63.54 kJ/mol RDX was estimated from these reaction rate coefficients at three different aquifer temperatures. Results of the radiolabel study demonstrated that the ultimate fate of RDX under in situ reductive conditions is highly dependent on redox conditions in the aquifer. In treatment columns (redox change, ΔE_h = -550 to -700 mV), 23-46 percent of initial radiocarbon was mineralized to $^{14}CO_2$ compared with <5 percent in control columns, where ΔE_h

(Continued)

15. SUBJECT TERMS

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[^{14}C]RDX
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In situ bioremediation
RDX
Reductive biotransformation

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ranged between 70 and -70 mV. The dissolved fraction of initial radiocarbon in treatment columns was estimated between 46 and 64 percent. No or very low levels of nitroso-substituted RDX transformation products were identified in dissolved fraction from treatment columns. In control columns dissolved fraction accounted for about 86 percent of initial ^{14}C and was composed of mainly untreated RDX.